From: (b)(6)
Sent: Tue, 9 Jun 2015 10:02:15 +0800
To: (b)(6) Chen, Ping (NIH/NIAID) [E]; (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E]; 危宏平
Subject: Re: a possible visit

Thanks

Dave

Sent from my BlackBerry 10 smartphone.

From: (b)(6)
Sent: Tuesday, June 9, 2015 8:51 AM
To: Chen, Ping (NIH/NIAID) [E]; (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E]; 危宏平
Subject: Re: RE: a possible visit

July 7 in the morning at 9:00 is OK for me. Zhengli.

SHI Zhengli, Ph. D
Senior Scientist & Professor
Wuhan Institute of Virology, Chinese Academy of Sciences
44 Xiao Hong Shan
430071 Wuhan, Hubei
China
Tel & Fax: (b)(6)
Email: (b)(6)

From: Chen, Ping (NIH/NIAID) [E]
Date: 2015-06-09 07:58
To: David T NHDe; (b)(6)
CC: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: a possible visit

Works for me.

Tuesday July 7 in the morning, 9 or 10 am?

Ping

Ping Chen, PhD
Director of NIAID Office in China
Office of Global Research, NIAID, NIH
Bethesda Office: (b)(6)
BB: (b)(6)
Beijing Office: (b)(6)
Cell: (b)(6)

U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: David T NHDe (b)(6)
Sent: Tuesday, June 09, 2015 6:50
To: Chen, Ping (NIH/NIAID) [E]; (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E]; David sr
Subject: Re: a possible visit

Dr Shi and Ping
Would it be possible to change to the manning of the 7th to meet... I just received notice of a meeting I must chair in Singapore with 3 agencies there?
It would be much better if I could leave the afternoon of 7th to travel to Singapore.
I can arrive on 6 July in afternoon to Wuhan if that helps.
Sorry just found out last night...
Thank you for your understanding.

Be well

Dave

Sent from my BlackBerry 10 smartphone.

From: Chen, Ping (NIH/NIAID) [E]
Sent: Monday, June 8, 2015 10:27 AM
To: (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: a possible visit

Dr. Shi,

Let's plan on a visit on July 8th. I plan to arrive at Wuhan on the evening of the 6. I have other NIAID grantees to visit. I will contact them soon. Based on everyone's schedule, I may have to make changes so I can get maximal use of my time for the trip.

I will follow up with more information later.

Thank you

Ping

Ping Chen, PhD
Director of NIAID Office in China
Office of Global Research, NIAID, NIH

Bethesda Office: (b)(6)
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Beijing Office: (b)(6)
Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: (b)(6)
Sent: Monday, June 08, 2015 9:58
To: Chen, Ping (NIH/NIAID) [E]
Cc: (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: a possible visit

Dear Dr. Chen,

It's nice to hearing from you. I'm happy to have a discussion with you when you visit Wuhan. May we make an appointment between July 7th to 9th?

Best regards,

Zhengli,

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Senior Scientist & Professor
Wuhan Institute of Virology, Chinese Academy of Sciences
44 Xiao Hong Shan
430071 Wuhan, Hubei
China
Tel & Fax: (b)(6)
Email: (b)(6)

From: Chen, Ping (NIH/NIAID) [E]
Date: 2015-06-08 09:34
To: (b)(6)
CC: Stemmy, Erik (NIH/NIAID) [E]
Subject: a possible visit

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Please let me know if the time works for you. If so when would be the best day for you. I will need to make my travel arrangement.

Thank you

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Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: (b)(6)
Sent: Mon, 8 Jun 2015 11:14:04 +0800
To: Chen, Ping (NIH/NIAID) [E]
Cc: (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: RE: a possible visit

0k, that's great!

SHI Zhengli, Ph. D
Senior Scientist & Professor
Wuhan Institute of Virology, Chinese Academy of Sciences
44 Xiao Hong Shan
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Date: 2015-06-08 10:27
To: (b)(6)
CC: Stemmy, Erik (NIH/NIAID) [E]
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Ping Chen, PhD
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Office of Global Research, NIAID, NIH
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Beijing Office: (b)(6)
Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: David T NHDe
Sent: Mon, 8 Jun 2015 11:02:46 +0800
To: Chen, Ping (NIH/NIAID) [E]; (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: a possible visit

Ping
I will make arrangements to arrive on 7th and stay at Marriott Renaissance Hotel.
Thanks
Dave

Sent from my BlackBerry 10 smartphone.

From: Chen, Ping (NIH/NIAID) [E]
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To: (b)(6)
Cc: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: a possible visit

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Zhengli,

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Beijing Office: (b)(6)
Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

From: (b)(6)
Sent: Mon, 8 Jun 2015 09:49:39 +0800
To: Chen, Ping (NIH/NIAID) [E]; (b)(6)
Cc: (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: a possible visit

Hi Shi
Hope you are well ...
I am available anytime on 7th -9th as I must be in manila the following week.

Hope to see you in July

Dave

Sent from my BLU Smartphone Device

"Chen, Ping (NIH/NIAID) [E]" (b)(6) wrote:

Dear Dr. Shi,

I am the NIAID representative working in Beijing. I and others from US gov. representatives planned to visit your institute in May (Hongping was our contact) but it was canceled. David Trudil suggested that I can contact you to schedule a visit. I actually had planned to do so as I want to visit the researchers in the Wuhan area who receive NIAID funding either through direct awards or through collaborations with NIAID awardees in US.

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Cell: (b)(6)
U.S. Embassy Beijing
#55 An Jia Lou Road
ChaoYang District, 100600
Beijing, China
(b)(6)

(b)(6)

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Fri, 1 Dec 2017 17:19:13 +0000
To: Denison, Mark
Cc: Baric, Ralph
Subject: RE: New CETR RFA

Not directly. Our Biodefense, Resources, and Translational Research office manages the CETR program.

Erik

From: Denison, Mark (b)(6)
Sent: Friday, December 01, 2017 12:05 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Baric, Ralph (b)(6)
Subject: Re: New CETR RFA

Thanks Erik. Yeah i saw it. Appreciate the note. Are you involved with it?

Mark.

Sent from my iPhone

On Dec 1, 2017, at 11:03 AM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Ralph and Mark,
You've probably already heard, but the new CETR RFA has been released. Thought I'd pass the link on in case you hadn't seen.

Erik

CETR RFA: https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-17-042.html

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email: (b)(6)

From: Smith, Philip (NIH/NIAID) [E]
Sent: Tue, 14 Jul 2015 15:10:53 -0400
To: Denison, Mark (NIH)
Cc: Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph; (b)(6)
Subject: RE: Grant Number: 5R01AI108197 - 03 PI Name: Denison, Mark R.

We actually need this as soon as possible. Please provide the document by no later than 7/16.

Philip Smith
☎: (b)(6)
✉:

From: Denison, Mark (NIH)
Sent: Tuesday, July 14, 2015 3:09 PM
To: Smith, Philip (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph; (b)(6)
Subject: Re: Grant Number: 5R01AI108197 - 03 PI Name: Denison, Mark R.
Importance: High

Dear Mr. Smith.
Thanks for the note and attention to the report.
I will review this. I am away all week at virology meeting.
I will try to get to you before then, but would early next week be acceptable for a response?

Regards

Mark Denison

From: <Smith>, "Philip [E] (NIH/NIAID)" (b)(6)
Date: Tuesday, July 14, 2015 1:53 PM
To: Mark Denison (b)(6)
Cc: "Degrace, Marciela (NIH/NIAID) [E]" (b)(6) "Stemmy, Erik (NIH/NIAID) [E]" (b)(6)
Subject: Grant Number: 5R01AI108197 - 03 PI Name: Denison, Mark R.

Good Afternoon,

In the above referenced progress report, it is noted that there is mention of 2 new publications that were a result of the work being done in reporting period 2. **Please provide a MyNCBI report reflecting these any other publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award. Please have this document routed through your signing official for concurrence.**

In the future, if there are publications or manuscripts you will need to answer “Yes” to C.1 and include a MyNCBI report of the relevant publications.

Thanks,

Philip Smith

Grants Management Specialist
Grants Management Program, DEA, NIAID, NIH
5601 Fishers Lane, Rm 4E24, MSC 9833 GMP
Rockville, Maryland 20892-9824
☎: (b)(6)
✉:

Effective October 1, 2014, NIH closeout policy has changed (see NOT-OD-14-084). In order to avoid unilateral closeout, final reports must be submitted in a timely manner. Failure to submit accurate final reports could result in enforcement actions such as revisions to NOA funding levels, or delay in future funding.

From: Wilburn, Shellie (NIH/NIAID) [E]
Sent: Tue, 4 Nov 2014 10:49:56 -0500
To: (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; (b)(6) Denison, Mark (NIH)
Subject: RE: Response: R01 AI 108197 - 02 - Mark Denison, PI

Thanks, if we have any further questions we will contact you.

Shellie Wilburn
Lead Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E43, MCS 9824
Bethesda, MD 20892-9824
Office Phone: (b)(6)
Email (b)(6)
E-Fax: 301-493-0597

"Coming together is a beginning. Keeping together is progress. Working together is success." - Henry Ford

---

From: (b)(6)
Sent: Monday, November 03, 2014 11:49 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Wilburn, Shellie (NIH/NIAID) [E]; Baric, Ralph; (b)(6) Denison, Mark (NIH); Sullivan, Donna (NIH/NIAID) [E]
Subject: RE: Response: R01 AI 108197 - 02 - Mark Denison, PI

Please find the attached response, from Dr. Mark Denison, to the October 22 letter regarding Gain of Function research. This is in regards to grant R01 AI 108197 - 02 and Dr. Denison's email below.

Should you have any questions or require additonal information please do not hesitate to contact us.

Thank You,
(b)(6)

From: Denison, Mark
Sent: Friday, October 31, 2014 10:19 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Wilburn, Shellie (NIH/NIAID) [E]; Ralph Baric; (b)(6)
Subject: Response: R01 AI 108197 - 02 - Mark Denison, PI

Dear Erik and Shellie,

Here is my letter of response to the otter of Oct 22 expressing concerns. I will send it through our grants / business office for their approval (cc'd above)

What is best, an email, from them approving it or do you need something signed by their office? Also I didn't know how many people (see below) need this notification. I am trusting you and Shellie can deliver this to the proper people.

Best Regards

Mark

Mark R. Denison M.D.
Craig-Weaver Professor of Pediatrics
Professor of Pathology, Microbiology & Immunology
Vanderbilt School of Medicine
D6217 MCN
Nashville, TN 37232-2581
(b)(6) (office)
(cell)
(b)(6)

From: "Sullivan, Donna (NIH/NIAID) [E]" (b)(6)
Date: Wednesday, October 22, 2014 12:00 PM
To: (b)(6)
Cc: Mark Denison (b)(6) "Glowinski, Irene (NIH/NIAID) [E]" (b)(6) "Kirker, Mary (NIH/NIAID) [E]" (b)(6) "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) "Wilburn, Shellie (NIH/NIAID) [E]" (b)(6)
Subject: 5 R01 AI 108197 - 02 - Mark Denison, PI

(b)(6)

Please see attached letter.

Thank you,

Donna

Donna R. Sullivan
Chief, Branch A
GMP, DEA, NIAID, NIH, DHHS
5601 Fishers Lane, Rm. 4G50, MSC 9824
Rockville, MD 20852 (For Express Mail: 20892-9824)
Tel: (b)(6)
Fax: 301-493-0597

From: (b)(6)
Sent: Mon, 3 Nov 2014 16:49:02 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Wilburn, Shellie (NIH/NIAID) [E]; Baric, Ralph; (b)(6) Denison, Mark (NIH); Sullivan, Donna (NIH/NIAID) [E]
Subject: RE: Response: R01 AI 108197 - 02 - Mark Denison, PI
Attachments: Denison AI108197 GOF Response.pdf

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Subject: 5 R01 AI 108197 - 02 - Mark Denison, PI

(b)(6)

Please see attached letter.

Thank you,

Donna

Donna R. Sullivan
Chief, Branch A
GMP, DEA, NIAID, NIH, DHHS
5601 Fishers Lane, Rm. 4G50, MSC 9824
Rockville, MD 20852 (For Express Mail: 20892-9824)
Tel: (b)(6)
Fax: 301-493-0597

Department of Pediatrics
Mark R. Denison, M.D.

VANDERBILT School of Medicine

October 26, 2014

Shellie Wilburn, Grants Management Specialist
Erik J. Stemmy, Ph.D. Program Officer
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

RE: Potential GOF concerns for 5 R01 AIO108197-02

Dear Dr. Stemmy and Ms. Wilburn

I am writing in response to the letter of Oct 21, 2014 concerning notification of concerns that Specific Aim 3 of the above referenced grant may contain gain-of-function studies that would be subject to the recently announced U.S. Government funding pause. *Please see the detailed response below.* I have reviewed these responses with my Co-PI, Ralph Baric Ph.D. who agrees with the responses.

Aim 3. To determine the effect of altered fidelity on in vivo replication and pathogenesis. In **part 1** we will use selected increased and decreased fidelity mutants to test replication and pathogenesis in mice. In **part 2**, we will determine minimal lethal dose, lung pathology, tissue tropism and effects on respiratory function in young and aged mice,. In **part 3,** we will test increased and decreased fidelity mutants during in vivo passage for genotypic and phenotypic stability. In **part 4** we will apply results from parts 1-3 in animal models of MERS-CoV to test conserved attenuating ExoN fidelity mutants on replication, pathogenesis, immune response and stability.

RESPONSE TO CONCERNS:

- **NO GOF studies planned or performed in this Aim or any Aims of AI 108197
- The Aim is designed to define the changes in pathogenesis, replication and disease associated with changes in fidelity. No experiments are designed to engineer increased pathogenicity or transmission. Further, the SARS mouse model is not a transmission model and no studies in the proposal will test that.
- Experiments in Parts 1-2 follow approach used in Graham et al and Menachery et al which showed that ExoN- and MT- mutants are less fit and are attenuated in vivo, with less pathology.
- Experiments in Part three follow approach in Graham et al which showed 1) that ExoN- genotype does not primarily revert and 2) that the attenuated mutator phenotype is not complemented or have reversion to WT virulence. This even after infection and passage of aged or immunocompromised mice.
- **We have under NO circumstances (*in vitro* or *in vivo*) found either WT or increased virulence or pathogenesis with any of our ExoN- or MT- mutant viruses. Thus our published data and experimental design has no GOF potential.
- Finally studies of pathogenesis are integral to any understanding of attenuation, and future development of new targets for antivirals and therapeutics. In fact our discovery that altered fidelity is Attenuating has resulted in this proposal to define determinants and another proposal to target ExoN by therapeutics designed to inhibit fidelity and render virus more susceptible to inhibition by lethal mutagenesis using RNA mutagens.

Regards

(b)(6)

Mark R. Denison M.D.
Craig-Weaver Professor of Pediatrics
Professor of Pathology, Microbiology & Immunology
Vanderbilt School of Medicine
D6217 MCN, Nashville, TN 37232-2581

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NOV 03 2014

(b)(6)

APPROVED

cc: Ralph Baric Ph.D.

From: Denison, Mark (NIH)
Sent: Fri, 31 Oct 2014 15:19:12 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Wilburn, Shellie (NIH/NIAID) [E]; Baric, Ralph; (b)(6)
Subject: Response: Response: R01 AI 108197 - 02 - Mark Denison, PI
Attachments: Denison AI108197 GOF response.pdf

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(b)(6) (office)
(cell)
(b)(6)

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Date: Wednesday, October 22, 2014 12:00 PM
To: (b)(6)
Cc: Mark Denison (b)(6) "Glowinski, Irene (NIH/NIAID) [E]" (b)(6) "Kirker, Mary (NIH/NIAID) [E]" (b)(6) "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) "Wilburn, Shellie (NIH/NIAID) [E]" (b)(6)
Subject: 5 R01 AI 108197 - 02 - Mark Denison, PI

(b)(6)

Please see attached letter.

Thank you,

Donna

Donna R. Sullivan
Chief, Branch A
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Department of Pediatrics
Mark R. Denison, M.D.

VANDERBILT School of Medicine

October 26, 2014

Shellie Wilburn, Grants Management Specialist
Erik J. Stemmy, Ph.D. Program Officer
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

RE: Potential GOF concerns for 5 R01 AIO108197-02

Dear Dr. Stemmy and Ms. Wilburn

I am writing in response to the letter of Oct 21, 2014 concerning notification of concerns that Specific Aim 3 of the above referenced grant may contain gain-of-function studies that would be subject to the recently announced U.S. Government funding pause. *Please see the detailed response below.* I have reviewed these responses with my Co-PI, Ralph Baric Ph.D. who agrees with the responses.

Aim 3. To determine the effect of altered fidelity on in vivo replication and pathogenesis. In **part 1** we will use selected increased and decreased fidelity mutants to test replication and pathogenesis in mice. In **part 2**, we will determine minimal lethal dose, lung pathology, tissue tropism and effects on respiratory function in young and aged mice,. In **part 3,** we will test increased and decreased fidelity mutants during in vivo passage for genotypic and phenotypic stability. In **part 4** we will apply results from parts 1-3 in animal models of MERS-CoV to test conserved attenuating ExoN fidelity mutants on replication, pathogenesis, immune response and stability.

RESPONSE TO CONCERNS:

- **NO GOF studies planned or performed in this Aim or any Aims of AI 108197
- The Aim is designed to define the changes in pathogenesis, replication and disease associated with changes in fidelity. No experiments are designed to engineer increased pathogenicity or transmission. Further, the SARS mouse model is not a transmission model and no studies in the proposal will test that.
- Experiments in Parts 1-2 follow approach used in Graham et al and Menachery et al which showed that ExoN- and MT- mutants are less fit and are attenuated in vivo, with less pathology.
- Experiments in Part three follow approach in Graham et al which showed 1) that ExoN- genotype does not primarily revert and 2) that the attenuated mutator phenotype is not complemented or have reversion to WT virulence. This even after infection and passage of aged or immunocompromised mice.
- **We have under NO circumstances (*in vitro* or *in vivo*) found either WT or increased virulence or pathogenesis with any of our ExoN- or MT- mutant viruses. Thus our published data and experimental design has no GOF potential.
- Finally studies of pathogenesis are integral to any understanding of attenuation, and future development of new targets for antivirals and therapeutics. In fact our discovery that altered fidelity is Attenuating has resulted in this proposal to define determinants and another proposal to target ExoN by therapeutics designed to inhibit fidelity and render virus more susceptible to inhibition by lethal mutagenesis using RNA mutagens.

Regards

(b)(6)

Mark R. Denison M.D.
Craig-Weaver Professor of Pediatrics
Professor of Pathology, Microbiology & Immunology
Vanderbilt School of Medicine
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cc: Ralph Baric Ph.D.

From: Denison, Mark (NIH)
Sent: Wed, 22 Oct 2014 17:11:59 +0000
To: Sullivan, Donna (NIH/NIAID) [E]; (b)(6)
Cc: Glowinski, Irene (NIH/NIAID) [E]; Kirker, Mary (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Wilburn, Shellie (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]; Baric, Ralph
Subject: Re: 5 R01 AI 108197 - 02 - Mark Denison, PI
Attachments: AI108197 - Denison[1].pdf

Dear Dr. Sullivan,

I was expecting the letter. I was in communication with my Program officer, Erik Stemmy , yesterday and have generated a response to the concern raised above. We already have published study in Nature Medicine showing that altered fidelity is stably and irrevocably attenuating in mouse models of SARS-CoV. Data in multiple other RNA viruses (including now influenza) supports that conclusion as well. But we will readdress the concerns cited and provide supporting published and preliminary data.

I will work with my Co-PI, Ralph Baric (UNC) to provide a common answer as the work on animals is performed at UNC, but is a joint project.

Now back to the NSABB GOF meeting webcast!

Regards

Mark Denison

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From: <Sullivan>, "Donna [E] (NIH/NIAID)" (b)(6)
Date: Wednesday, October 22, 2014 12:00 PM
To: (b)(6)
Cc: Mark Denison (b)(6) "Glowinski, Irene (NIH/NIAID) [E]" (b)(6) "Kirker, Mary (NIH/NIAID) [E]" (b)(6) "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) "Wilburn, Shellie (NIH/NIAID) [E]" (b)(6)
Subject: 5 R01 AI 108197 - 02 - Mark Denison, PI

(b)(6)

Please see attached letter.

Thank you,

Donna

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
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Bethesda, Maryland 20892

October 21, 2014

(b)(6)
Vanderbilt University
Office of Sponsored Programs
1400 18th Avenue South
Nashville, TN 37212-2809

RE: 5 R01 AI108197-02

Dear (b)(6)

NIAID has determined that the above referenced grant may include Gain of Function (GoF) research that is subject to the recently-announced U.S. Government funding pause (http://www.phe.gov/s3/dualuse/Documents/gain-of-function.pdf), issued on October 17, 2014. The following specific aims appear to involve research covered under the pause:

Aim 3: To determine the effect of altered fidelity on *in vivo* replication and pathogenesis.

As your grant is currently funded, this pause is voluntary. Organizations conducting GoF research supported by the NIH have an opportunity to transition the applicable research to research that is not covered by the funding pause; halt the applicable GoF research until the outcome of the deliberative process is known; or continue to conduct the applicable GoF research until the end of the currently active budget period.

NIAID requests information on Vanderbilt University's plans for the research outlined above within 90 days of the date of this letter.

- **If you determine that the above research does NOT include GoF work subject to the funding pause**, please provide a detailed explanation of the research being conducted and why it is not covered by the pause. NIAID will review this information and make the final determination.
- **If the ongoing research includes GoF work subject to the funding pause and the grantee proposes to transition it to areas of research not covered by the pause,** please provide the transition plan. It should identify the research to be transitioned, a detailed description of the

new planned specific aims (in most cases this will require NIAID pre-approval), and a timeline for the proposed transition.

- **If the grantee plans to voluntarily halt the research subject to the funding pause**, please identify the research that will be halted and the proposed date by which the applicable research will be stopped. Please provide a confirmation that the research has been halted.
- **If the ongoing research includes GoF work and the grantee plans on continuing the research until the end of the currently active budget period,** please provide a detailed description of the GoF research to be conducted.

These plans are for the currently active budget period. Please be advised that while the funding pause is in effect, NIAID will not support GoF research identified in the pause after the end of the current grant budget period. Neither competing nor non-competing renewal applications will be funded to support applicable GoF research.

If you have any questions about this matter please do not hesitate to contact the NIAID program and/or grants management contact listed below.

(b)(6)

Grants Management Specialist
NIAID/NIH/DHHS

(b)(6)

Erik J. Stemmy, Ph.D.
Program Officer
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

CC: Dr. Mark Dennison
Ms. Mary Kirker
Dr. Irene Glowinski

From: Denison, Mark (NIH)
Sent: Tue, 21 Oct 2014 20:27:01 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: Baric, Ralph; (b)(6) Sims, Amy C; (b)(6) (b)(6) Beanan, Maureen (NIH/NIAID) [E]; (b)(6)
Subject: AI108197- No GOF - response to discussion.
Attachments: Graham Nature Medicine.pdf, Smith PLoS Path 2013.pdf

Dear Erik,

Thank you for your helpful discussion today. I have added text below in blue to respond to your questions and clarify the areas that might be of concern. Please let me know if you have any additional questions or clarifications needed. As per our discussion, I am copying to Maureen Beanan.
Regards, Mark

These are the current aims of **AI 108197 Determinants of replication fidelity.**

Aim 1. To define nsp14 fidelity determinants and their impact on SARS-CoV and MERS-CoV replication and fitness. In **part 1,** we will use MHV and SARS-CoV to test the effect of predicted and systematic mutations in nsp14-ExoN motifs and residues, Zn finger domain, conditional (ts) alleles, conserved charged residues outside of the ExoN motif, and the carboxy-terminal N7-methyltransferase domain in nsp14 on replication fidelity by next generation sequencing and mutagen sensitivity. Experiments in **part 2** will test the impact of altered fidelity on virus genotypic and phenotypic stability and competitive fitness during infection and passage in culture. In **part 3** we will use the newly established reverse genetic system for **MERS-CoV** to test for conservation of ExoN mediated fidelity and fidelity altering mutations on replication in multiple continuous and primary cell lines of the human lung.

Comments:

- ****NO GOF studies planned or performed in this AIM**
- All experiments in Aim 1 done in vitro in tissue culture
- To experiments with MHV and SARS-CoV nsp14 exoN- mutants (lacking proofreading) have: 1) replication defect 2) profound loss of fitness compared to WT virus (Graham Nature medicine 2013) 3) inability to revert the ExoN genotype or compensate the mutator phenotype
- ExoN- mutants are profoundly more sensitive to RNA mutagens compared to wild type virus (Smith et al Plos pathogens 2013)
- ExoN- mutants >100 fold more sensitive to IFN than WT (Brett Case, Denison lab unpublished)
- No mutations in nsp14 have demonstrated increased replication or fitness.

Aim 2. To define the effect of nsp14-ExoN fidelity altering mutations on RNA synthesis, and on exonuclease and N7-methyltransferase activity in vitro. In **part 1**

we will determine the effect of increased and decreased fidelity mutations on RNA synthesis and recombination **for SARS-CoV and MERS-**CoV. In **part 2,** we will determine the in vitro biochemical mechanism of activity of altered fidelity mutations in vitro on nsp14 Exonuclease and N7-methyltransferase activity. In **part 3** we will determine the sensitivity of nsp14 mutants to RNA mutagens, nucleoside analogs and β-IFN, testing the mechanism action during infection.

Comments:

- ****NO GOF studies planned or performed in this AIM**
- It has not yet been possible to recover ExoN- mutants of MERS-CoV. We believe that the lower level replication of MERS-CoV, combined with replication and decreased fitness of ExoN minus makes recovery difficult. We thus predict impaired fitness of ExoN- MERS
- MT- mutants are less fit and are attenuated (Menachery 2014, Baric Lab). Our preliminary data shows nsp14-N7MT- mutant is less fit, has replication defect and is profoundly sensitive to IFN. (Brett Case unpublished).

Aim 3. To determine the effect of altered fidelity on *in vivo* replication and pathogenesis. We will test the *hypothesis that decreased or increased fidelity is attenuating for SARS-CoV and MERS-CoV replication and pathogenesis in vivo, while allowing protective immune response*. In **part 1** we will use selected increased and decreased fidelity mutants to test replication and pathogenesis in aged, immunocompromised and persistently infected mice of different genetic backgrounds. In **part 2,** we will determine minimal lethal dose, lung pathology, tissue tropism and effects on respiratory function in young and aged mice, in order to define the limits of fidelity regulation on in vivo pathogenesis in the lung. **In part 3,** we will test increased and decreased fidelity mutants during in vivo passage for genotypic and phenotypic stability and reversion to virulence. In **part 4** we will apply results from **parts 1-3** in animal models of **MERS-CoV** to test conserved attenuating ExoN fidelity mutants on replication, pathogenesis, immune response and stability.

Comments:

- ****NO GOF studies planned or performed in this Aim**
- Experiments in Parts 1-2 follow approach used in Graham et al and Menachery et al which showed that ExoN- and MT- mutants are less fit and attenuated, with less pathology.
- Experiments in Part three follow approach in Graham et al which showed that ExoN- genotype does not primarily revert and attenuated mutator phenotype is not complemented or have reversion to WT virulence. This even after infection and passage of Aged, or immunocompromised mice, or passage in SCID mice.

- Experiments in Part 3 follow approach in Graham et al which showed that ExoN- genotype does not primarily revert and attenuated mutator phenotype is not complemented or have reversion to WT virulence. ****We have under NO circumstances (in vitro, in vivo) found either WT or increased virulence or pathogenesis with any of**

our ExoN- or MT- mutant viruses. Thus our published data and experimental design has no GOF potential.

- This approach is critical to define the stability of the changes, to define possible viral and cellular interacting proteins, and to define mechanism of action in replication

Regards

Mark

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nature medicine

A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease

Rachel L Graham[1], Michelle M Becker[2], Lance D Eckerle[2], Meagan Bolles[3], Mark R Denison[2,4] & Ralph S Baric[1,3]

Live, attenuated RNA virus vaccines are efficacious but subject to reversion to virulence. Among RNA viruses, replication fidelity is recognized as a key determinant of virulence and escape from antiviral therapy; increased fidelity is attenuating for some viruses. Coronavirus (CoV) replication fidelity is approximately 20-fold greater than that of other RNA viruses and is mediated by a 3′→5′ exonuclease (ExoN) activity that probably functions in RNA proofreading. In this study we demonstrate that engineered inactivation of severe acute respiratory syndrome (SARS)-CoV ExoN activity results in a stable mutator phenotype with profoundly decreased fidelity *in vivo* and attenuation of pathogenesis in young, aged and immunocompromised mice. The ExoN inactivation genotype and mutator phenotype are stable and do not revert to virulence, even after serial passage or long-term persistent infection *in vivo*. ExoN inactivation has potential for broad applications in the stable attenuation of CoVs and, perhaps, other RNA viruses.

Of the approximately 335 emerging infectious diseases that were identified between 1940 and 2004, 60.3% originated in wildlife[1]. From past pandemics, it is clear that highly pathogenic zoonoses are major threats to global human health, economic stability and national security[1–4]. SARS-CoV and swine influenza virus CA/04/09 H1N1 have caused substantial human morbidity and mortality in the 21st century. Similar to influenza, CoVs have a strong history of host shifting and cross-species transmission[5,6]. In addition to the emergence of SARS-CoV in 2002, which caused 50% mortality in aged populations, several other human CoVs, such as HCoV-NL63, HCoV-OC43 and HCoV-229E, probably emerged from animal reservoirs within the past 200 years[7,8]. The sudden emergence of new respiratory viral pathogens from animals underscores the need for new, broadly applicable vaccine strategies that rapidly and rationally attenuate emerging zoonoses, especially to protect vulnerable populations in future outbreaks.

Vaccines have a long history of success in reducing viral disease burdens. Live, attenuated viruses are ideal vaccine candidates, as they elicit balanced innate and adaptive lifelong protective immune responses with low production and delivery costs[9]. Unfortunately, broadly applicable strategies for the rational design of live, attenuated virus vaccines have remained elusive, and vaccines attenuated by chemical treatment or passage can revert to virulence, resulting in disease outbreaks in unvaccinated and immunocompromised populations[9]. Moreover, the precise mechanism of attenuation often remains unclear; thus, the stability of the attenuation cannot be clearly evaluated or assured.

RNA viruses encode RNA-dependent RNA polymerases that lack efficient proofreading capabilities; the resulting high error rates, which range from 10^{-3} to 10^{-5} mutations per site per round of replication, render RNA viruses highly vulnerable to lethal mutagenesis using chemical agents[10,11]. High mutation rates generate considerable genomic diversity, allowing RNA viruses to rapidly adapt to changing environmental conditions and hosts[12]. Increased replication fidelity has been shown to reduce the virulence of poliovirus and chikungunya virus[12–14] and has been proposed as a strategy for live, attenuated virus design[15]. CoVs encode the largest known RNA virus genomes (26–32 kb), exceeding the theoretical limits of viable RNA genome size[11]. Mutation rates are lower in CoVs than in other RNA viruses, approaching 2×10^{-6} mutations per site per round of replication[16]. Nsp14, encoded in the viral replicase gene, contains a 3′→5′ exoRNase (ExoN) of the DEDDh exonuclease superfamily[17]. In addition to the CoVs, ExoN homologs are present in the members of the Nidovirales order, whose genomes are >20 kb, but are not present in the smaller arteriviruses (with genomes of 12–16 kb), suggesting that the ExoN had a crucial role in genome expansion[16,18]. *In vitro*, 3′→5′ exoRNase activity has been demonstrated for recombinant SARS-CoV nsp14 (ref. 19). We have engineered and recovered viable ExoN inactivation mutants from mouse hepatitis virus (MHV-ExoN) and SARS-CoV (Urbani background, SARS-ExoN). Both MHV-ExoN and SARS-ExoN inactivations are maintained stably for more than ten passages

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in vitro and have 15- to 20-fold increased mutation frequencies compared to wild-type MHV and SARS-CoV[16,20]. Thus, ExoN has a crucial role in CoV RNA genome replication fidelity *in vitro*, probably by directly mediating or stimulating proofreading, a function previously unknown among RNA viruses[21].

In this study, we used the stable, low-fidelity mutator phenotype of the SARS-CoV ExoN mutants to determine whether decreased replication fidelity could be used as a rational design strategy for a live, attenuated vaccine with broad potential applications to other viruses[16,20,21]. We evaluated (i) the impact of the inactivation of an RNA-proofreading exonuclease and the resultant mutator phenotype on CoV replication, fitness and pathogenesis; (ii) virus stability after passage or persistence *in vivo*; and (iii) the efficacy of using a decreased-fidelity mutant as a vaccine. Further, we assessed the potential for generating stably attenuated, reversion-resistant, immunogenic strains of known and newly identified CoVs to be used as vaccines in both immunocompetent and immunocompromised populations.

RESULTS

The mutator phenotype and decreased fitness of MA-ExoN

We engineered nsp14 ExoN inactivation mutations into the background of the virulent mouse-adapted SARS-CoV (MAwt), yielding MA-ExoN (**Fig. 1a**,**b**). We compared MAwt, which causes increased mortality and acute respiratory distress in young and aged mouse models[22–24], and MA-ExoN in *in vitro* growth experiments (multiplicity of infection (MOI) = 0.1 PFU per cell). MA-ExoN showed a stable growth defect of less than 1 log (**Fig. 1c**). When placed in direct competition, MA-ExoN was clearly less fit than MAwt over successive rounds of infection (**Supplementary Fig. 1a**,**b**). At 6 h after infection (p.i.), MA-ExoN genome RNA levels were roughly equivalent to those of MAwt and were lower than those of MAwt at 12 h p.i.; by 24 h p.i., MA-ExoN genome RNA levels were approximately 10% of those of MAwt (**Supplementary Fig. 1c**). Thus, the data suggest that MA-ExoN is able to initiate and establish replication efficiently through times of peak RNA synthesis (0–6 h) but has impaired accumulation, which manifests late in one round of infection and is amplified over multiple rounds. These results are consistent with accumulating defects resulting from a markedly increased mutation rate (see the Discussion section).

We then sequenced RNA from multiple MAwt and MA-ExoN plaques. Both the MAwt background and engineered ExoN mutations were present in all sequenced MA-ExoN clones. Additionally, MA-ExoN accumulated 14-fold more unique mutations and had a mean 11.5-fold greater mutation frequency compared to MAwt (P <0.01) (**Fig. 1d**,**e**). These results confirm that the growth and replication fidelity impairments of the nsp14 ExoN mutator phenotype are present in MA-ExoN and are indistinguishable from those in SARS-ExoN during replication in culture.

MA-ExoN is attenuated *in vivo*

To assess MA-ExoN virulence, we infected young (10-week-old) and aged (14-month-old) female BALB/c mice with MA-ExoN or MAwt (**Fig. 2**). Young mice infected with MAwt showed dose-dependent weight loss and recovery (**Fig. 2a**), though they had no observable dose-dependent differences in lung titers or clearance after day 4 p.i. (**Fig. 2b**). In contrast, young mice infected with MA-ExoN showed no signs of clinical disease and had high but not dose-dependent lung titers that were rapidly cleared by day 4 p.i. (**Fig. 2a**,**b**). We then compared MA-ExoN and MAwt infection in aged, immunosenescent mice[25]. Mice infected with either virus experienced dose-dependent weight loss (**Fig. 2c**); however, although lung titers were equivalent across all doses of MA-ExoN and MAwt on day 2 p.i., mice infected with MA-ExoN cleared the virus independent of inoculation dose, whereas mice infected with MAwt had begun to clear the virus from higher-titer infections more efficiently at day 4 p.i. than from lower-titer infections (**Fig. 2d**). Additionally, whereas aged mice infected with MA-ExoN had no mortality, MAwt-infected mice had dose-dependent mortality (**Fig. 2e**). As described previously[23], we found little if any virus in other organs. These experiments demonstrate that MA-ExoN is attenuated in both young and aged diseased mice compared to virulent MAwt and that disease symptoms, when present, are less pronounced in MA-ExoN infections than MAwt infections.

A potential concern with live, attenuated vaccines is the chance that they could revert to virulence *in vivo*, particularly in immunocompromised individuals. Therefore, we assessed whether MA-ExoN was

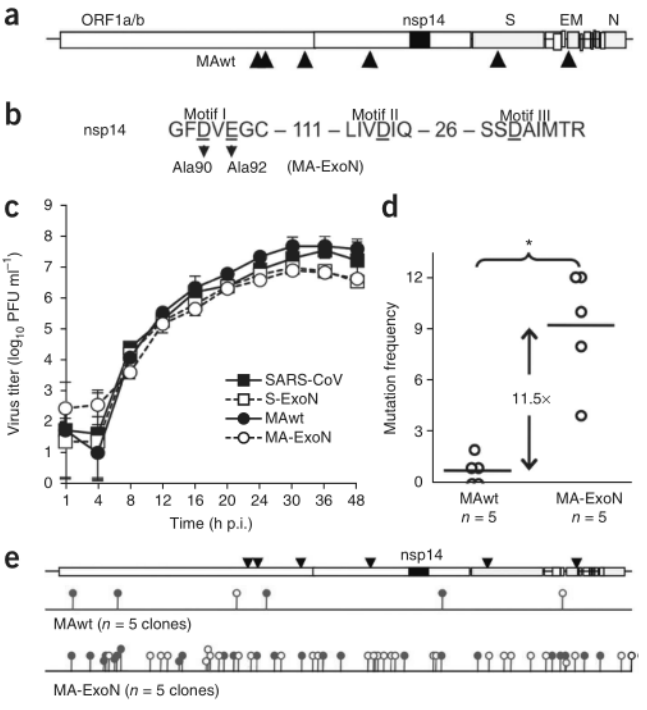

Figure 1 The nsp14 ExoN mutator virus in a virulent mouse-adapted SARS-CoV isogenic background. (**a**) Genome organization, with the locations of the nsp14 coding sequence (black rectangle) and the mouse-adapted mutations (triangles) shown. ORF1a/b, ORF1a and ORF1b. Structural proteins are labeled as follows: S, spike; E, envelope; M, membrane; N, nucleocapsid. (**b**) Nsp14 ExoN motifs, DEDD domain residues (underlined) and alanine substitutions (D90A and E92A) in motif I recovered in wild-type SARS-CoV and MAwt backgrounds. (**c**) Growth analysis (MOI = 0.1 PFU per cell) of wild-type SARS-CoV, S-ExoN, MAwt and MA-ExoN on Vero cells. Error bars, s.d. (**d**) Mutation frequency from complete genome sequencing of plaque isolates of MAwt and MA-ExoN (n = 5 for both) at passage 3. The increase in mean mutation frequency (horizontal lines) in MA-ExoN compared to MAwt (11.5×) is indicated. *P < 0.01 (Mann-Whitney nonparametric test for independent samples). (**e**) The mutations identified with complete genome sequencing across five clones from each group. Filled circles, nonsynonymous mutations; open circles, synonymous mutations; black, noncoding mutations; red, mutations present in more than one clone; blue, mutations present in only one clone. Mouse-adapted mutations are shown as triangles on the genome schematic and were present in all sequenced genomes.

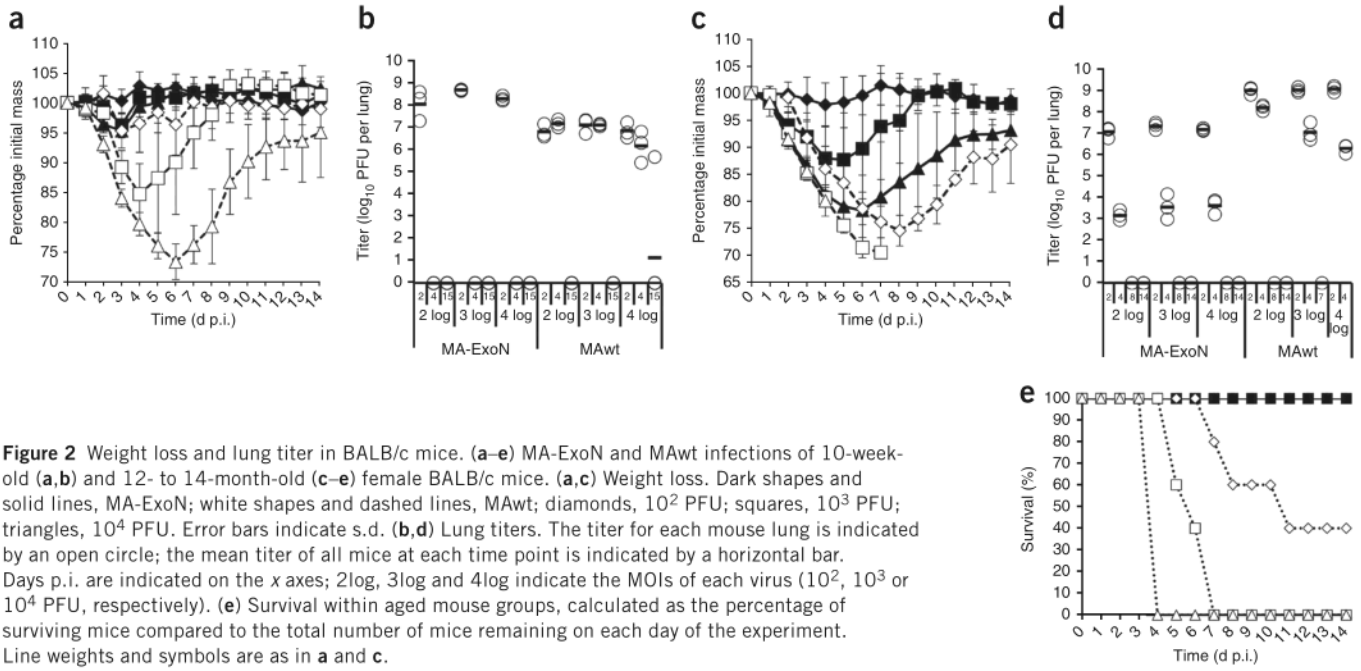

Figure 2 Weight loss and lung titer in BALB/c mice. (**a**–**e**) MA-ExoN and MAwt infections of 10-week-old (**a**,**b**) and 12- to 14-month-old (**c**–**e**) female BALB/c mice. (**a**,**c**) Weight loss. Dark shapes and solid lines, MA-ExoN; white shapes and dashed lines, MAwt; diamonds, 10^2 PFU; squares, 10^3 PFU; triangles, 10^4 PFU. Error bars indicate s.d. (**b**,**d**) Lung titers. The titer for each mouse lung is indicated by an open circle; the mean titer of all mice at each time point is indicated by a horizontal bar. Days p.i. are indicated on the *x* axes; 2log, 3log and 4log indicate the MOIs of each virus (10^2, 10^3 or 10^4 PFU, respectively). (**e**) Survival within aged mouse groups, calculated as the percentage of surviving mice compared to the total number of mice remaining on each day of the experiment. Line weights and symbols are as in **a** and **c**.

attenuated in immunocompromised mice. We used MAwt and MA-ExoN to infect young *Rag*$^{-/-}$ (recombination activating gene), severe combined immunodeficiency (SCID) and *Stat1*$^{-/-}$ (signal transducer and activator of transcription 1) mice, as well as background controls (C57BL/6, BALB/c and 129 mice, respectively). All MA-ExoN–infected mice had significantly less weight loss than MAwt-infected mice ($P < 0.05$; **Fig. 3a**–**c** and **Supplementary Table 1**). Only *Stat1*$^{-/-}$ mice had any notable weight loss (~15%) as a result of MA-ExoN infection; however, these mice did not pass experimental morbidity thresholds (**Fig. 3c**). In contrast, all MAwt-infected *Stat1*$^{-/-}$ mice died or were moribund by day 9 p.i., but MAwt infection was not lethal in C57BL/6 or 129 control mice (**Fig. 3a**,**c**), as has been previously reported[22,26]. *Rag*$^{-/-}$ and SCID mice maintained detectable amounts of MAwt and MA-ExoN virus for 14 d (*Rag*$^{-/-}$) or 60 d (SCID) beyond the background controls (**Fig. 3d**,**e**) but showed no signs of illness over the course of the experiment despite a lack of viral clearance, expanding earlier reports from our laboratory that MAwt does not clear from *Rag*$^{-/-}$ mice[26]. The rapid clearance of MA-ExoN infection from *Stat1*$^{-/-}$ mice (**Fig. 3f**) further supports the hypothesis that clearance of SARS-CoV infection is dependent on both B and T cells[27].

Mutation accumulation during persistent *in vivo* infection

Infection with both MAwt and MA-ExoN persisted for at least 60 d in SCID mice (**Fig. 3e**), potentially allowing for the most longitudinal

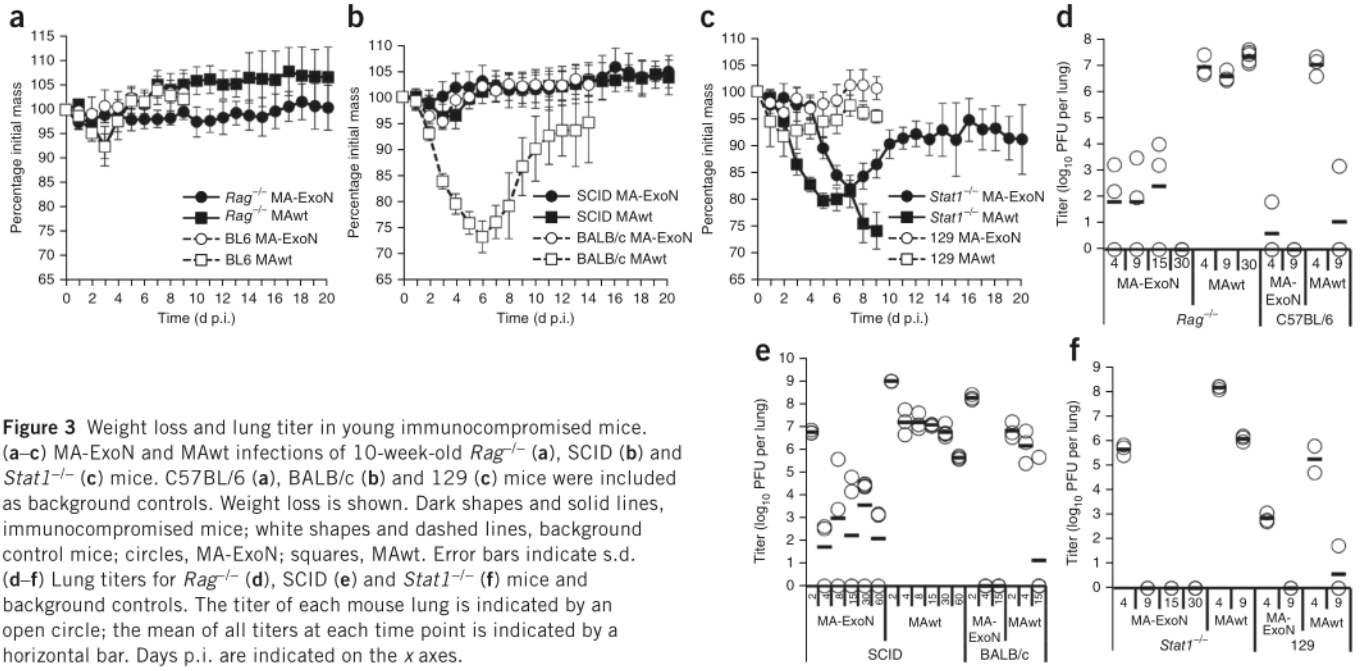

Figure 3 Weight loss and lung titer in young immunocompromised mice. (**a**–**c**) MA-ExoN and MAwt infections of 10-week-old *Rag*$^{-/-}$ (**a**), SCID (**b**) and *Stat1*$^{-/-}$ (**c**) mice. C57BL/6 (**a**), BALB/c (**b**) and 129 (**c**) mice were included as background controls. Weight loss is shown. Dark shapes and solid lines, immunocompromised mice; white shapes and dashed lines, background control mice; circles, MA-ExoN; squares, MAwt. Error bars indicate s.d. (**d**–**f**) Lung titers for *Rag*$^{-/-}$ (**d**), SCID (**e**) and *Stat1*$^{-/-}$ (**f**) mice and background controls. The titer of each mouse lung is indicated by an open circle; the mean of all titers at each time point is indicated by a horizontal bar. Days p.i. are indicated on the *x* axes.

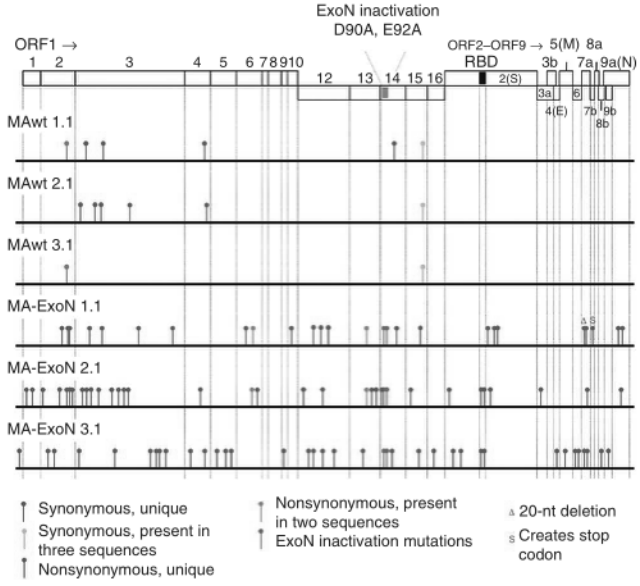

Figure 4 Mutation accumulation in infected SCID mice at 30 d p.i. The SARS-CoV genome is depicted at the top. The nsp14 ExoN coding region is denoted by a purple box, with the inactivating amino acid changes indicated above the schematic. The receptor-binding domain (RBD) is denoted by a black box. Individual SCID mouse genome sequences are represented by black horizontal lines. Dashed lines separate the nonstructural protein sequences in ORF1 and downstream ORFs. Mutations are indicated by lollipop shapes colored as follows: blue, synonymous, unique to one sequence; light blue, synonymous, present in three sequences; red, nonsynonymous, unique to one sequence; green, synonymous, present in two sequences; purple, nsp14 ExoN inactivation mutations. Mutations that alter the size of an ORF are indicated by a red Δ (deletion) or a red S (stop codon). Genome sizes, ORF and nonstructural protein boundaries and mutation marker placements are approximate.

cycles of replication and lowest immune barriers to the emergence of mutations conferring increased fitness, reversion to virulence and fidelity-compensating changes of any of the groups examined. To test this, we sequenced viral genomes from viral plaques grown from SCID mouse lung homogenates at 30 d p.i. (**Fig. 4** and **Supplementary Table 2**). We identified a total of 14 consensus mutations (~100,000 nt) for MAwt, with 3 mutations shared in two or three genomes, resulting in 11 distinct mutations (4 synonymous and 7 nonsynonymous). For MA-ExoN, the engineered inactivation mutations were maintained. In contrast to MAwt, MA-ExoN plaques contained a total of 91 mutations (89 distinct: 32 synonymous and 57 nonsynonymous), corresponding to a 9.6-fold higher total mutation accumulation compared to MAwt.

We compared mutation accumulations across two separate regions (open reading frame 1a (ORF1a), nucleotides 493–8603, and ORF1b, nucleotides 12915–16520; **Supplementary Figs. 2** and **3** and **Supplementary Table 2**) for statistical determinations. Mutation accumulations were significantly higher in MA-ExoN–infected mice for both regions ($P < 0.01$). Additionally, there was a mean 18.3-fold increased accumulation for MA-ExoN across the ORF1a region. When we normalized the accumulations of mutations per 10 kb, MA-ExoN mutation accumulations in the ORF1a compared to the ORF1b region were not significantly different ($P = 0.340$) but remained significantly increased compared to the accumulation in MAwt ($P < 0.001$ for both ORF1a and ORF1b). We identified no new

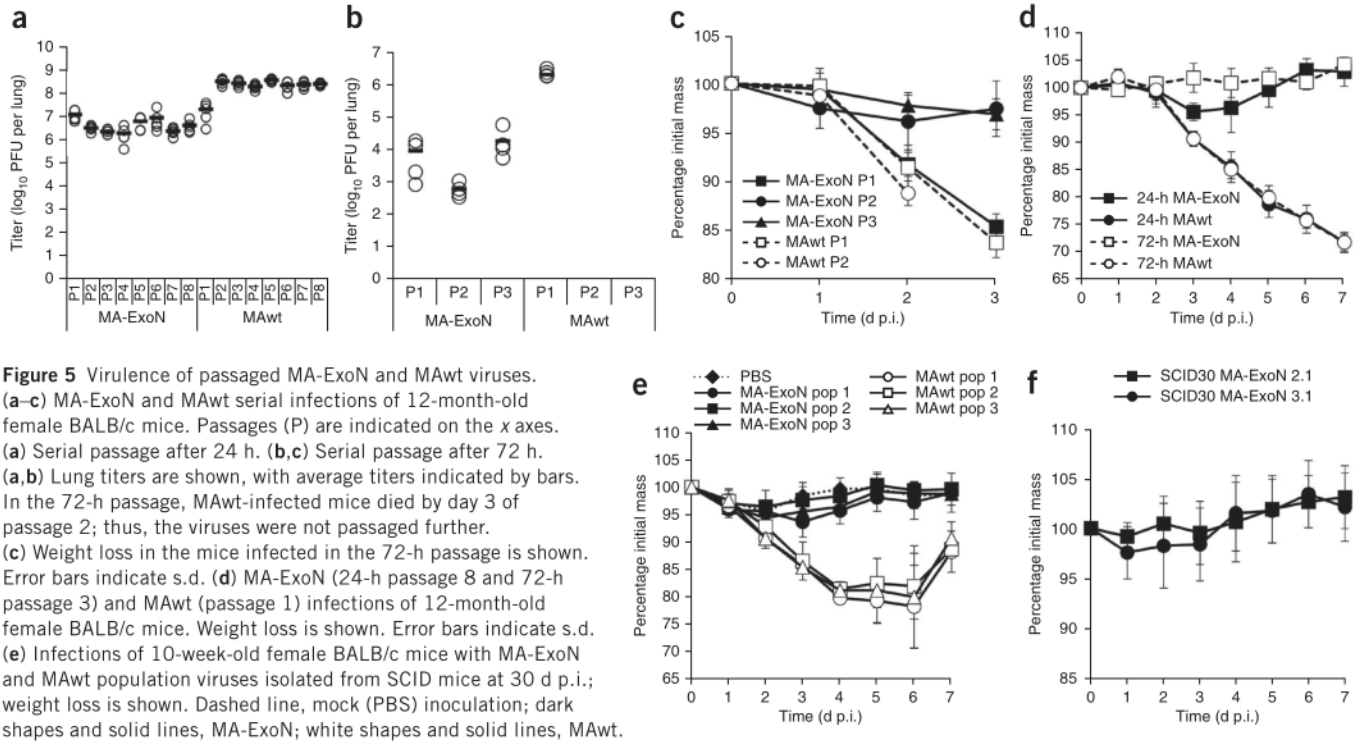

Figure 5 Virulence of passaged MA-ExoN and MAwt viruses. (**a**–**c**) MA-ExoN and MAwt serial infections of 12-month-old female BALB/c mice. Passages (P) are indicated on the *x* axes. (**a**) Serial passage after 24 h. (**b**,**c**) Serial passage after 72 h. (**a**,**b**) Lung titers are shown, with average titers indicated by bars. In the 72-h passage, MAwt-infected mice died by day 3 of passage 2; thus, the viruses were not passaged further. (**c**) Weight loss in the mice infected in the 72-h passage is shown. Error bars indicate s.d. (**d**) MA-ExoN (24-h passage 8 and 72-h passage 3) and MAwt (passage 1) infections of 12-month-old female BALB/c mice. Weight loss is shown. Error bars indicate s.d. (**e**) Infections of 10-week-old female BALB/c mice with MA-ExoN and MAwt population viruses isolated from SCID mice at 30 d p.i.; weight loss is shown. Dashed line, mock (PBS) inoculation; dark shapes and solid lines, MA-ExoN; white shapes and solid lines, MAwt. For each virus, lungs from three separate mice were harvested (mouse 1–mouse 3), and viruses were subsequently inoculated without plaque purification (population); circles, mouse 1 populations (pop 1); squares, mouse 2 populations (pop 2); triangles, mouse 3 populations (pop 3). (**f**) Weight loss in 11-month-old female BALB/c mice infected with MA-ExoN 2.1 (with C16999A) or MA-ExoN 3.1 (without C16999A) plaque isolates from SCID mice after 30 d of infection (SCID30). Squares, SCID30 MA-ExoN 2.1; circles, SCID30 MA-ExoN 3.1. Error bars indicate s.d.

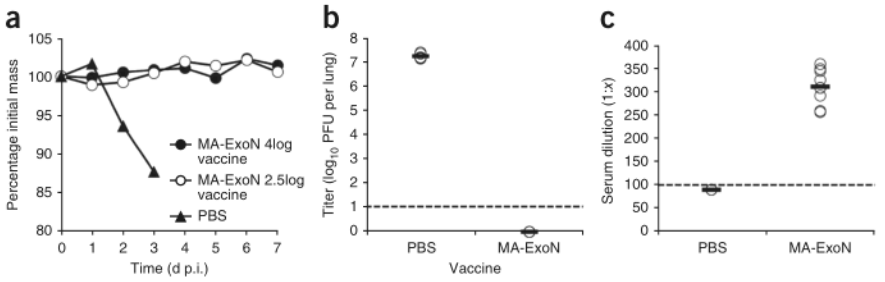

Figure 6 MA-ExoN vaccination protects from lethal challenge. (**a**,**b**) Low-passage MA-ExoN and mock (PBS) vaccinations of 12-month-old female BALB/c mice followed by lethal challenge with MAwt. (**a**) Weight loss in challenged mice. Dark circles, 10^4 PFU MA-ExoN vaccination; white circles, $10^{2.5}$ PFU MA-ExoN vaccination; dark triangles, PBS vaccination. Error bars indicate s.d. (**b**) Lung titers at day 2 after challenge. ExoN was given at a 2.5log ($10^{2.5}$ PFU) vaccination. The mean titer is indicated by a bar in each group. (**c**) Fifty-percent plaque reduction neutralization titer ($PRNT_{50}$) assay using sera from PBS-vaccinated and MA-ExoN–vaccinated mice to neutralize MAwt. Reciprocal dilutions capable of effecting 50% plaque reduction are shown by circles; mean reciprocal dilutions are indicated by a bar for each group. The limit of detection for each assay, if given, is indicated by a dashed line.

mutations in any of the three MA-ExoN plaques, suggesting no obligatory or consistent pattern of adaptation or mutational bias. The most prevalent mutation identified (C16999M) was also present in viral stocks as a polymorphism; however, its frequency in the viral population remained stable (~40%) both *in vitro* and *in vivo* and in experiments with both BALB/c and SCID mice (**Supplementary Tables 2** and **3** and data not shown). The results from persistent ExoN infection over 30 d were consistent with the results from passage of SARS-ExoN virus in culture: the ratios of accumulation of unique mutations (MA-ExoN:MAwt) during replication in SCID mice ranged from ~9.6:1 to 18.3:1, which is similar to the ratio measured between SARS-ExoN and SARS-CoV in culture[16].

MA-ExoN resists reversion to virulence *in vivo*

To test the resistance to reversion to virulence during passage *in vivo*, we subjected MAwt and MA-ExoN to both short and long serial passages (24 h and 72 h per passage, respectively) in aged BALB/c mice (**Fig. 5**). In both groups, viral titer remained stable from passage to passage (**Fig. 5a**,**b**); additionally, viral plaque phenotypes were preserved, and the ExoN-inactivating mutations and amino acid substitutions were maintained (data not shown). In contrast, whereas MAwt titer remained stable over the 24-h serial passage, mice inoculated with lung homogenates from the 72-h passage died by day 3 in passage 2 (**Fig. 5b**). Although MA-ExoN titer remained stable over the 72-h passage, mouse weights did not decrease during infection after passage 1 (**Fig. 5c**). Notably, there was no evidence of a gain of virulence with serial passage in the MA-ExoN pathogenesis model: when aged mice were infected with lung homogenates from each of the final 24-h and 72-h passages, mice infected with MA-ExoN passages lost no or little weight, and MAwt-infected mice became moribund (**Fig. 5d**).

To test whether persistent MA-ExoN infection could result in phenotypic reversion to virulence, we used viruses harvested from SCID mice at day 30 p.i. to infect young BALB/c mice (**Fig. 5e**). MAwt-infected mice showed signs of morbidity (weight loss, hunched posture and ruffled fur) but recovered. In contrast, MA-ExoN–infected mice showed no clinical signs of illness, as in the initial infection (**Fig. 2a**). Additionally, plaques containing different mutational subsets were identically attenuated after re-infection of BALB/c mice (**Fig. 5f**). These results demonstrate that after 30 d of persistent infection, the ExoN mutator phenotype did not revert to virulence despite the greatly increased mutation rate and population diversity.

MA-ExoN vaccination protects mice from lethal challenge

Aged mice mount poor productive immune responses to SARS-CoV vaccines and remain highly susceptible to severe disease and lethal infection[24,28], thus representing the most sensitive means to measure vaccine efficacy against lethal SARS-CoV infection. To test the efficacy of the MA-ExoN mutant as a possible vaccine against lethal challenge, we vaccinated aged BALB/c mice with MA-ExoN and allowed them to recover from infection. We then challenged the mice with a lethal dose of MAwt (**Fig. 6**). Mock-vaccinated mice succumbed to MAwt challenge by day 3 p.i. and had high lung titers (**Fig. 6a**,**b**); however, mice vaccinated with MA-ExoN were protected from illness (**Fig. 6a**). Further, in contrast to other vaccine platforms[24,28,29], MA-ExoN–vaccinated mice had no detectable lung titers 2 d after challenge (**Fig. 6b**).

Additionally, mice vaccinated with MA-ExoN generated high amounts of neutralizing antibodies (mean, 1:311 ± 37.5 reciprocal 50% neutralization titer) (**Fig. 6c**). The minimal neutralizing titers for protection against SARS-CoV infection in mice have been reported as 1:25–1:49 (ref. 30). Thus, even with a single vaccination, MA-ExoN provided complete protection against lethal challenge in a susceptible, immunosenescent mouse model of lethal SARS-CoV infection. To our knowledge, the MA-ExoN virus is the first approach to SARS-CoV immunization that fully protects against clinical disease and viral replication in an aged mouse model[24,28,29].

DISCUSSION

Live, attenuated vaccines have substantially reduced the global disease burden associated with viral infections, including, for example, those of measles, mumps, rubella, polio, yellow fever and chickenpox[9,15]. However, live, attenuated vaccines carry several risks, including primary or secondary reversion to a virulent phenotype, as in the case of poliovirus[9]. In fact, attenuated phenotypes encounter natural selective pressures for reversion that can cause outbreaks of disease in unvaccinated populations[31].

RNA virus replication fidelity has evolved to balance genome diversity and stability; therefore, inactivating an enzyme, such as nsp14-ExoN, that is responsible for high-fidelity replication could theoretically drive the virus toward instability, deleterious mutational diversity and decreased fitness in complex environments, which we observed here *in vitro*. It is possible that nsp14-ExoN may have other functions in viral RNA synthesis[21]; however, global impairment of viral RNA synthesis alone cannot explain the *in vitro* results and the attenuating phenotype *in vivo*.

It is not possible to fully separate defects caused by increased mutation load from those resulting from the replication defect observed, and both probably contribute to the phenotype. However, our results are consistent with the hypothesis that both stable and evolving defects resulting from the mutator phenotype have irrevocably attenuated MA-ExoN. These defects could include (i) mutations that impair or terminate translation, replication and transcription;

(ii) mutations that impair or abolish protein functions; or (iii) changes in RNA polymerase processivity in the presence of an inactivated proofreading exonuclease. These combined impairments may be manifested with CoV ExoN mutants, as the high number of accumulated mutations per genome is unprecedented among viral mutator strains. Indeed, the phenotype observed with CoV ExoN inactivation is similar to those reported for other polymerase complexes with inactivated exonucleases, such as human mitochondrial DNA polymerase γ (pol γ)[32,33] and bacteriophage T7 DNA polymerase[34–36]. In pol γ studies, the loss of proofreading was associated with impaired polymerase activity in a manner that was probably causal, impossible to uncouple and characterized by decreased speed, increased template dissociation and restricted access of nucleotides to the polymerase active site[32,33]. For CoVs, the high levels of iterative amplification of both genomic and subgenomic RNA would further accelerate these deleterious processes by providing aberrant templates. The loss of ExoN proofreading would continuously generate new potentially attenuating alleles, and defective genomes and would reduce both genome fitness and the risks for primary and secondary reversions to virulence.

In this study, we demonstrate that MA-ExoN is attenuated in mice and that the mutant clears rapidly in the presence of an adaptive immune response. Although our *in vivo* experiments with MAwt recapitulated many of the phenotypes observed in aged and immunocompromised human populations, additional testing of MA-ExoN as a vaccine in primates will be necessary to further confirm its stability *in vivo*[37]. Experiments in SCID mice with persistent MA-ExoN infection verified the accumulation of mutations across the genome without evidence for the selection of either phenotypic virulence-enhancing alleles or primary genotypic reversion. Viruses harvested after passage remained avirulent, supporting the conclusions that (i) selection for virulence is not occurring; (ii) selection is being outcompeted by the gradual accumulation of attenuating mutations in individual genomes or the population mutational swarm; or (iii) the mutant is unable to generate or select for either fast-growing or slow-clearing viruses that are also more virulent. Not surprisingly, we identified a limited number of polymorphisms in the MA-ExoN virus stock that we used in subsequent experiments. Viruses with and without these mutations were fully attenuated *in vivo*, and the mutations were also maintained in lungs in the same frequencies as in the virus stock, suggesting that they were not selected against during passage *in vivo*. Thus, no single mutation or polymorphism could be clearly linked to viral attenuation except for the ExoN inactivation.

Notably, we have shown that MA-ExoN vaccinations are completely protective against replication and lethal challenge in aged BALB/c mice, the SARS-CoV mouse pathogenesis model that captures most of the severe clinical disease outcomes in human infections. Additionally, neutralization titers were equivalent or superior to those reported in studies of two-dose alphavirus replicon S glycoprotein vaccines and killed vaccines containing alum, with the additional advantage of protecting against virus replication and clinical disease[24,28,29].

Live, attenuated vaccines must have two characteristics, aside from the capacity to elicit a protective immune response: resistance to primary reversion and stable attenuation at secondary sites. We have demonstrated that MA-ExoN has both of these characteristics. In all circumstances, the engineered inactivation mutations were maintained, indicating that exonuclease activity is not crucial for the virus life cycle and that the 4-nt, 2–amino acid change presents a substantial barrier to primary reversion; further, the passage experiments suggest that the virus lacks redundant or complementing mechanisms to fully restore the loss of ExoN activity. In addition, MA-ExoN harvested from persistently infected SCID mice retained an attenuated phenotype when re-inoculated into young BALB/c mice, suggesting that persistence does not select for virulence. Future studies are necessary to address whether additional modifications could enhance and stabilize the attenuated phenotype by reducing the likelihood of gain of function by homologous recombination, such as introduction of the ExoN inactivation in a background with rewired transcriptional regulatory sequences[38], which could increase resistance to reversion.

The inactivation of putative viral proofreading components in the pursuit of a stable vaccine constitutes a paradigm that has high potential to be broadly applicable to those members of the Nidovirales order with an exonuclease activity. In a time when metagenomics studies inform us of the likelihood of future viral emergence events—viruses that have the potential to afflict the human population much as SARS-CoV did in 2002–2003—the design and ready implementation of an attenuation strategy that can be rapidly applied to any emerging CoV potentially represents a major advance in the preservation of public health. These data should also encourage the pursuit of fidelity-impairing mutations in the replicase proteins of other RNA viruses as potential targets or the use of CoVs as vaccine vectors for rational vaccine design.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Plaque-purified MA-ExoN isolate sequences are deposited in GenBank under the following accession codes: FJ882942; FJ882943; FJ882945; FJ882948; FJ882951; FJ882952; FJ882953; FJ882957; FJ882958; FJ882959; FJ882961; FJ882962; HQ890526; HQ890527; HQ890528; HQ890529; HQ890530; HQ890531; HQ890532; HQ890533; HQ890534; HQ890535; HQ890536; HQ890537; HQ890538; HQ890539; HQ890540; HQ890541; HQ890542; HQ890543; HQ890544; HQ890545; HQ890546; JF292902; JF292903; JF292904; JF292905; JF292906; JF292907; JF292908; JF292909; JF292910; JF292911; JF292912; JF292913; JF292914; JF292915; JF292916; JF292917; JF292918; JF292919; and JF292920.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

R.L.G. designed and performed experiments, analyzed data, and wrote and edited the paper. M.M.B., L.D.E. and M.B. performed experiments, analyzed data and read the paper. M.R.D. and R.S.B. designed experiments, analyzed data, and wrote and edited the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Construction of SARS D plasmid with ExoN and mouse-adapted mutations. A SARS-CoV D plasmid was constructed by restriction digestion and ligation of existing SARS D–ExoNI[16] and SARS D mouse-adapted[23] plasmids. Briefly, both plasmids were restriction digested with BstB I and Xba I enzymes. After treatment of the digested SARS D–ExoNI plasmid with Antarctic Phosphatase (New England BioLabs, Ipswitch, MA, USA), fragments were isolated, purified and ligated together using T4 DNA Ligase overnight at 4 °C as described previously[39]. Colonies were screened for proper insert size by restriction digestion and electrophoresis, and the presence of the appropriate mutations was verified by sequencing.

Generation of SARS-CoV MA-ExoN mutant virus. Virus containing the ExoNI inactivation and mouse-adapted mutations within the viral coding sequence was produced using the infectious complementary DNA (cDNA) assembly strategy for SARS-CoV as previously described[39,40]. ExoN viruses were kept at low passage (one passage past virus rescue, P1) to minimize the accumulation of mutations in cell culture. For this study, an equivalently low-passaged MAwt virus was used for comparison.

***In vitro* passage series and viral growth and plaque assays.** MAwt and MA-ExoN viruses were grown in Vero cells at MOI = 0.1 PFU per cell in all *in vitro* experiments, with the exception of the genome RNA quantification experiment, which was performed at MOI = 3 PFU per cell. Passage series and growth experiments were performed, and viral titers were determined as previously described[16].

Competition assay. Vero cells were plated at 10^6 cells per well in six-well plates. Cells were infected at an MOI of 0.1 with combinations of MAwt and MA-ExoN at 1:1, 1:10 and 1:100 ratios favoring either MA-ExoN or MAwt and were incubated for 24 h at 37 °C. After 24 h, 100-µl aliquots of each supernatant were passed to fresh six-well plates of Vero cells for five total successive passages, and infected monolayers were harvested in TRIzol (Invitrogen, Grand Island, NY, USA). After passages were complete, RNA was purified according to the manufacturer's protocol, and first-strand cDNA was generated as described[41]. PCR products were produced using the primers S32F and S34R (**Supplementary Table 4**). Once the presence of single-band PCR products was verified by agarose gel electrophoresis and the yields were calculated by spectrometry, 100 ng of each product was restriction digested using BsrF I, which cuts in the nsp14-ExoN engineered mutation site but not in the corresponding MAwt sequence. Digested products were resolved on a 1.7% agarose gel, and normalized relative percentages of MAwt and ExoN-MA digested products were calculated using ImageJ (http://rsbweb.nih.gov/ij/).

Quantification of genome RNA. Vero cells were infected with either MAwt or MA-ExoN at MOI = 3 PFU per cell. At 6, 12 and 24 h p.i., RNA was harvested in TRIzol and isolated according to the manufacturer's protocol. First-strand cDNA was generated as described above, and real-time PCR was performed assessing for genome RNA using the primers 5′-AGCCAACCAACCTCGATCT CTTGT-3′ (forward) and 5′-TGACACCAAGAACAAGGCTCTCCA-3′ (reverse). cDNA was normalized using the GAPDH primers 5′-TGCACCACCAA CTGCTTAGC-3′ (forward) and 5′-GGCATGGACTGTGGTCATGAG-3′ (reverse)[42]. Normalized results were then compared as ratios of MA-ExoN to MAwt genomes using the ΔΔCt method.

Infection of mice with SARS-CoV MAwt and MA-ExoN. All experimental protocols involving mice were reviewed and approved by the institutional animal care and use committee at the University of North Carolina, Chapel Hill. The following groups of mice were used: 10-week-old female BALB/c (Charles River Laboratories, Wilmington, MA, USA), 14-month-old female BALB/c (Harlan Laboratories, Indianapolis, IN, USA), 10-week-old female *Stat1*$^{-/-}$ (Taconic Farms, Hudson, NY, USA; stock 002045-M-F), 10-week-old female 129S6/SvEvTac (Taconic; stock 129SVE-F), 10-week-old female *Rag*$^{-/-}$ (Jackson Labs, Bar Harbor, ME, USA; stock 002216), 10-week-old female C57BL/6 (Jackson; stock 00064) and 10-week-old female SCID (Jackson; stock 001803). Mice were lightly anesthetized and infected intranasally with varying doses (10^2–10^4 PFU, depending on the experiment) of SARS-CoV MAwt or SARS-CoV MA-ExoN. Mice were weighed daily, and on certain days specified in each experiment, a subset of mice in each group was euthanized, and their lungs were harvested for virus titer. Mice that dropped below 70% of their initial mass or were moribund were euthanized before their scheduled time points. Serial passages were inoculated as above for passage 1; subsequent passages were inoculated with 50 µl of clarified lung homogenate (lungs were homogenized in 1 ml of PBS) from the previous passage. All experiments used $n = 5$ mice per virus per dosage per condition (if applicable) per time point, with the exception of experiments using immunocompromised mice, in which $n = 3$.

Determination of virus titer in infected mouse lungs. Lungs harvested for virus titer were weighed and homogenized in 1.0 ml PBS at 6,000 r.p.m. for 60 s in a MagnaLyser (Roche, Basel, Switzerland). Virus titers were determined by plaque assay on Vero cells as previously described[39].

Determination of viral neutralization antibody titers in mouse sera. Mouse sera were heat inactivated for 30 min at 55 °C and then serially diluted to 1:100, 1:200, 1:400, 1:800 and 1:1,600 in PBS to a volume of 125 µl. Next, 125 µl of PBS containing low-concentration MAwt (40 PFU) or high-concentration MAwt (240 PFU) was added to each serum dilution. The virus-serum mixtures were incubated at 37 °C for 30 min. After incubation, virus titers of the mixtures were determined by plaque assay as described[39]. We then calculated the $PRNT_{50}$ values, the serum dilutions at which plaque formation was reduced by 50% relative to that of virus stock not treated with serum.

Viral genome sequencing. To determine the sequences of viral genomes present in SCID mouse lungs after 30 d of infection, plaques were isolated from lung samples from SCID mice at 30 d p.i. as described above. Briefly, once individual, well-resolved viral plaques were visible, they were harvested by collecting the agarose plugs above them using a 200-µl pipette tip. Each agarose plug was dropped in 0.5 ml PBS, allowed to diffuse for 24 h at 4 °C and then applied to ~70% confluent monolayers of Vero cells in T25 flasks and incubated for 48 h at 37 °C. Infected cell monolayers were then harvested in 1 ml TRIzol. First-strand cDNA was generated as described[41]. Amplicons of the viral genomes were generated as follows: for whole-genome sequencing (amplicons 1–13) and partial-genome sequencing (amplicons A–G, X and Y), the primer pairs indicated in **Supplementary Table 4** were used in a 50-µl PCR reaction using Phusion polymerase (New England BioLabs). Five microliters of each PCR reaction were electrophoresed on agarose gels to verify the presence of correctly sized amplicons, and PCR products were purified using a Qiagen PCR Purification Kit (Qiagen, Valencia, CA, USA). Amplicons were then sequenced using the corresponding primer sets for each amplicon, as indicated in **Supplementary Table 4**. Sequence results were analyzed using Geneious Pro 5.3.6 (Biomatters, Auckland, New Zealand) and Serial Cloner 2.1 (SerialBasics, http://serialbasics.free.fr/Home/Home.html).

Statistical analyses. Statistical analyses were performed using the Mann-Whitney *U* test (http://elegans.som.vcu.edu/~leon/stats/utest.html). Significance was set at $P < 0.05$.

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Coronaviruses Lacking Exoribonuclease Activity Are Susceptible to Lethal Mutagenesis: Evidence for Proofreading and Potential Therapeutics

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Abstract

No therapeutics or vaccines currently exist for human coronaviruses (HCoVs). The Severe Acute Respiratory Syndrome-associated coronavirus (SARS-CoV) epidemic in 2002–2003, and the recent emergence of Middle East Respiratory Syndrome coronavirus (MERS-CoV) in April 2012, emphasize the high probability of future zoonotic HCoV emergence causing severe and lethal human disease. Additionally, the resistance of SARS-CoV to ribavirin (RBV) demonstrates the need to define new targets for inhibition of CoV replication. CoVs express a 3′-to-5′ exoribonuclease in nonstructural protein 14 (nsp14-ExoN) that is required for high-fidelity replication and is conserved across the CoV family. All genetic and biochemical data support the hypothesis that nsp14-ExoN has an RNA proofreading function. Thus, we hypothesized that ExoN is responsible for CoV resistance to RNA mutagens. We demonstrate that while wild-type (ExoN+) CoVs were resistant to RBV and 5-fluorouracil (5-FU), CoVs lacking ExoN activity (ExoN−) were up to 300-fold more sensitive. While the primary antiviral activity of RBV against CoVs was not mutagenesis, ExoN− CoVs treated with 5-FU demonstrated both enhanced sensitivity during multi-cycle replication, as well as decreased specific infectivity, consistent with 5-FU functioning as a mutagen. Comparison of full-genome next-generation sequencing of 5-FU treated SARS-CoV populations revealed a 16-fold increase in the number of mutations within the ExoN− population as compared to ExoN+. Ninety percent of these mutations represented A:G and U:C transitions, consistent with 5-FU incorporation during RNA synthesis. Together our results constitute direct evidence that CoV ExoN activity provides a critical proofreading function during virus replication. Furthermore, these studies identify ExoN as the first viral protein distinct from the RdRp that determines the sensitivity of RNA viruses to mutagens. Finally, our results show the importance of ExoN as a target for inhibition, and suggest that small-molecule inhibitors of ExoN activity could be potential pan-CoV therapeutics in combination with RBV or RNA mutagens.

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Introduction

The potential for CoVs to cause significant human disease is well demonstrated, with six known HCoVs—HKU1, OC43, NL63, 229E, SARS-CoV and MERS-CoV—causing colds, pneumonia, systemic infection, and severe or lethal disease [1–5]. Four of these viruses have been identified in just the last 10 years, with two, SARS-CoV and MERS-CoV, causing lethal respiratory and systemic infection [1,3–6]. Studies over the past 10 years have expanded the known phylogenetic, geographic, and species diversity of CoVs, and support multiple emergence events of CoVs into humans from bats and other zoonotic pools [7–10]. The most recent evidence for CoV trans-species movement comes from the emergence of the novel MERS-CoV [1,11,12]. From April 2012 to June 2013 MERS-CoV has caused 72 laboratory confirmed cases and up to 50% mortality from severe respiratory and systemic disease in at least 8 countries, with evidence for human-to-human transmission [13]. MERS-CoV is most closely related to the bat CoVs HKU4 and HKU5 [11], and the recently identified receptor dipeptidyl peptidase 4 (DPP4) is present on both human and bat cells [14], providing a compelling argument that zoonotic CoV infections resulting in severe human disease may be more frequent events than previously thought. Because of the lack of epidemiological data, it remains unknown whether multiple introductions from a zoonotic source or human transmission of a mild or asymptomatic disease is responsible for these continuing cases of sporadic severe infections. However, based on the high mortality rates associated with SARS-CoV and those reported for MERS-CoV [13], this novel virus potentially represents a serious threat to global health for which no vaccines or therapeutics currently exist.

CoVs contain the largest known RNA genomes (27–32 kb) and encode an array of 16 viral replicase proteins, including a 3′-to-5′ exoribonuclease (ExoN) domain within nonstructural protein 14

Author Summary

RNA viruses have high mutation rates (10^{-3} to 10^{-5} mutations/nucleotide/round of replication), allowing for rapid viral adaptation in response to selective pressure. While RNA viruses have long been considered unable to correct mistakes during replication, CoVs such as SARS-CoV and the recently emerged MERS-CoV are important exceptions to this paradigm. All CoVs encode an exoribonuclease activity in nonstructural protein 14 (nsp14-ExoN) that is proposed to prevent and/or remove misincorporated nucleotides. Because of the demonstrated resistance of SARS-CoV to the antiviral drug ribavirin (RBV), we hypothesized that ExoN is responsible for CoV resistance to RNA mutagens. Using RBV and the RNA mutagen 5-fluorouracil (5-FU), we show that CoVs lacking ExoN activity (ExoN−) are highly susceptible to RBV and 5-FU, in contrast to wild-type (ExoN+) CoVs. The inhibitory activity of 5-FU against ExoN− viruses resulted specifically from 5-FU incorporation during viral RNA synthesis that lead to extensive mutagenesis within the viral population, and was associated with a profound decrease in virus specific infectivity. These results demonstrate the proofreading activity of ExoN during virus replication and suggest that inhibitors of ExoN activity could be broadly useful inhibitors of CoV replication in combination with RBV or RNA mutagens.

(nsp14) [2,15–17]. Similar to the proofreading subunit (ε) of *E. coli* DNA polymerase III, CoV nsp14-ExoN is a member of the DEDD superfamily of DNA and RNA exonucleases [15,18]. This superfamily contains four conserved D-E-D-D acidic residues that are required for enzymatic activity, and mutation of these critical residues within CoV ExoN ablates or significantly reduces ExoN activity [15]. Studies from our group have demonstrated that ExoN activity is essential for high-fidelity replication in both the model CoV murine hepatitis virus (MHV) and SARS-CoV [19,20]. Inactivation of ExoN activity due to alanine substitution of the first two active site residues results in 15- to 20-fold reduced replication fidelity in cell culture [19,20] and a 12-fold reduction during SARS-CoV infection *in vivo* [21], associated with profound and stable attenuation of SARS-CoV virulence and replication. A recent study has shown that bacterially-expressed SARS-CoV nsp14-ExoN can remove mismatched nucleotides *in vitro*, and that ExoN activity is stimulated *in vitro* through interactions with the non-enzymatic CoV protein nsp10 [22]. Thus all bioinformatic, genetic and biochemical studies to date support the hypothesis that nsp14-ExoN is the first identified proofreading enzyme for an RNA virus and functions together with other CoV replicase proteins to perform the crucial role of maintaining CoV replication fidelity.

Retrospective clinical studies during the SARS epidemic ultimately concluded that treatment with ribavirin (RBV), an antiviral drug shown to be mutagenic for some RNA viruses [23,24], was ineffective against SARS-CoV [25–28]. Because ExoN activity is required for CoV high-fidelity replication [19–21], we sought to determine if ExoN was responsible for CoV resistance to RNA mutagens. Using the nucleoside analog RBV and the base analog 5-fluorouracil (5-FU; [29]) we show that CoVs lacking ExoN activity (ExoN−) are up to 300-fold more sensitive to inhibition than wild-type CoVs (ExoN+). Additionally, using full-genome next-generation sequencing we show that ExoN− viruses accumulate 15- to 20-fold more A:G and U:C transitions, consistent with 5-FU incorporation during RNA synthesis. Ultimately our results suggest the exciting possibility that small-molecule inhibitors of ExoN activity could be potential pan-CoV therapeutics, especially when used in combination with RBV or RNA mutagens.

Materials and Methods

Cell culture and viruses

Murine astrocytoma delayed brain tumor cells (DBT cells) were grown at 37°C and maintained in DMEM (Invitrogen) containing 10% FBS, supplemented with penicillin, streptomycin, HEPES, and amphotericin B. VeroE6 (Vero) cells were grown at 37°C and maintained in MEM (Invitrogen) containing 10% FBS supplemented with penicillin, streptomycin, and amphotericin B. All work with MHV was performed using the reverse genetics infectious clone based on strain MHV-A59 [30], and work with SARS-CoV was performed using the reverse genetics infectious clone based on the Urbani strain [31]. Viral studies using SARS-CoV were performed in Select Agent certified BSL-3 laboratories using protocols reviewed and approved by the Institutional Biosafety Committee of Vanderbilt University and the Centers for Disease Control for the safe study and maintenance of SARS-CoV.

Compounds and cell viability studies

5-fluorouracil (5-FU), ribavirin (RBV), guanosine (GUA) and mycophenolic acid (MPA) were obtained from Sigma. 5-FU and RBV were made as 200 mM stock solutions, and were prepared in DMSO and sterile water, respectively. GUA and MPA were prepared in DMSO as 40 mM or 100 mM stocks, respectively. Low concentration (μM) working stocks were prepared as needed in sterile water prior to dilution in DMEM. Viability of DBT and Vero cells was assessed using CellTiter-Glo (Promega) in 96-well plate format according to manufacturer's instructions. DBT and Vero cells were seeded into opaque tissue culture grade 96-well plates, and DMEM containing RBV or 5-FU was added to each well to achieve the concentrations indicated. Water or DMSO vehicle controls were performed, in addition to a 20% ethanol control for cell death. The cells were then incubated at 37°C for either 12 or 24 h, and cell viability was determined using a Veritas Microplate Luminometer (Promega). The resultant values were then normalized to untreated cells.

Drug sensitivity studies and plaque assays

Subconfluent monolayers of DBT cells in 6-well plates were pretreated for 30 min at 37°C with 1 mL of DMEM containing vehicle or the indicated concentration of RBV, 5-FU, MPA, or GUA. The drug was then removed and cells were infected with MHV-ExoN+ or ExoN− viruses at an MOI of 1 plaque forming units (PFU)/cell (single-cycle) or 0.01 (multi-cycle) for 30 min at 37°C. Virus was then removed and 1 mL of DMEM containing vehicle, RBV, 5-FU, MPA, or GUA was added to each well. Cells were then incubated at 37°C for either 12 (single-cycle) or 24 (multi-cycle) h. The supernatant was harvested and virus titer was determined by plaque assay on DBT cells. For SARS-CoV studies, subconfluent monolayers of Vero cells in T25 flasks were pretreated for 30 min at 37°C with DMEM containing vehicle, RBV, or 5-FU. The drug was removed and cells were infected with either SARS-ExoN+ or ExoN− viruses at an MOI of 0.1 PFU/cell (single-cycle) for 30 min. The virus was removed and DMEM containing vehicle, RBV, or 5-FU was added back. Cells were then incubated for 24 h, at which point the supernatant was harvested and virus titer was determined by plaque assay on Vero cells. All treated samples were normalized to the untreated vehicle control, and values were expressed as fold change from untreated virus titers.

Real-time quantitative reverse transcription PCR (real-time qRT-PCR) of viral genomic RNA

Viral RNA was harvested from infected cell monolayers using TRIzol reagent (Invitrogen), and was reverse transcribed (RT) using SuperScript III (Invitrogen). Random hexamers (1 μL of 50 μM stock) and 1 μg of total RNA were incubated for 5 min at 70°C. The remaining reagents were then added according to the manufacturer's protocol, and the mixture was incubated at 50°C for 1 h and then at 85°C for 5 min. All RT reactions were performed in a final volume of 20 μL. Real-time qRT-PCR was performed on the RT product using the Applied Biosciences 7500 Real-Time PCR System with Power SYBR Green PCR Master Mix (Life Technologies). Each reaction was performed in a total volume of 25 μL containing 12.5 μL of the Power SYBR Green PCR Master Mix, 125 ng each of the forward and reverse primers and 1 μL of the RT product which was diluted 1:1000. Viral genomic RNA was detected using primers (forward: ACAGGGTGGAGTTCCCGTTA and reverse: ACGGAAG-CACCACCATAAGA) optimized to generate a ~120 nt portion of ORF1a. These values were normalized using the $2^{-\Delta\Delta Ct}$ method [32] to endogenous expression of the housekeeping gene glyceraldehyde-3- phosphate dehydrogenase (GAPDH) using primers (forward: GGGTGTGAACCACGAGAAAT and reverse: CCTTCCACAATGCCAAAGTT) optimized to yield a ~120 nt portion of GAPDH [33,34]. Triplicate wells of each sample were analyzed, and averaged into one value representing a single replicate to minimize well-to-well variation. The cycle parameters were as follows: Stage 1, (1 rep) at 50°C for 2 min; Stage 2, (1 rep) 95°C for 10 min; Stage 3, (40 reps) at 95°C for 15 sec and 57°C for 1 min. One representative product from each treatment was verified by melting curve analysis and agarose gel electrophoresis.

Amplicon preparation for deep sequencing of whole viral genomes

Viral RNA from SARS-ExoN+ or ExoN− infected Vero monolayers was harvested using TRIzol reagent, and was reverse transcribed (RT) using SuperScript III as described above except with 5 μL of random hexamers (50 μM stock), 5 μg of total RNA, and in a final volume of 100 μL for each reaction. Four microliters of RT product was then used to generate 12 overlapping ~3 kb amplicons for each virus treated with either 0 or 400 μM 5-FU by PCR. The high-fidelity polymerase Easy A (Agilent) was used to ensure that errors were minimal during PCR. All primer sets generated single bands which were then purified using the Wizard SV Gel and PCR Clean-Up System (Promega).

Illumina next generation sequencing and analysis

Prior to sequencing, cDNA amplicons were fragmented (Fragmentase, NEB), clustered, and sequenced with Illumina cBot and GAIIX technology as previously described [35]. Between 1.4×10^8 and 4.5×10^8 bases, comprised of ~69-nt reads, were obtained per virus, and CASAVA 1.8.2 was used to demultiplex and create the fastq files. Low quality bases from the ends of each sequence read were then trimmed, using *Phred* scores as the guiding metric (error probabilities higher than 0.001), and sequences with less than 16 bases after trimming were discarded to reduce false alignment and subsequent false variant calls. The program fastq-clipper (http://hannonlab.cshl.edu/fastx_toolkit/index. html) was used for this quality filtering. The Burrows-Wheeler Alignment tool was then used to align reads to the SARS-CoV ExoN+ or ExoN− reference genomes with a maximum of two mismatches per read [36]. Base calling at each position was determined using SAMTOOLS [37]. After the pileup, an in-house script collected the data per-position. For each position throughout the viral genome, the bases and their qualities were gathered, each variant allele's rate was initially modified according to its covering read qualities based on a maximum likelihood estimation and test for significance using Wilks' theorem. Additionally, an allele confidence interval was calculated and output for each allele. Only alleles with statistically significant $p<0.05$ values were retained and considered to be true variants. Above 0.01% all variants were found to be statistically significant, while below 0.01% many variants could not be distinguished from background error. Thus, the background noise caused by sequencing error was determined to be 0.01% or less.

Statistical analysis

Statistical tests were applied where noted within the figure legends and were determined using GraphPad Prism (La Jolla, CA) software. Statistical significance is denoted (*$P<0.05$, **$P<0.01$, ***$P<0.0001$) and was determined using an unpaired, two-tailed Student's *t* test compared to either untreated samples or to the corresponding ExoN+ sample. For the cell viability studies, treated samples were compared to the DMEM sample containing DMSO.

Results

MHV-ExoN− viruses have increased sensitivity to RBV

Because RBV has been shown to be incorporated as ribavirin monophosphate (RMP) into viral RNA during replication [23,24,38–42], the presence of a proofreading enzyme would be predicted to exclude and/or remove nucleotide misincorporation [43–47]. If ExoN is responsible for the resistance phenotype, viruses lacking ExoN activity (ExoN−) should demonstrate increased titer reduction following RBV treatment as compared to wild-type viruses containing ExoN activity (ExoN+). To test this hypothesis, we examined the sensitivity of MHV-ExoN+ and ExoN− viruses to RBV during single-cycle (MOI = 1 PFU/cell) replication in murine astrocytoma delayed brain tumor cells (DBT cells). No toxicity was observed in DBT cells following treatment with up to 400 μM RBV (Figure 1A). MHV-ExoN+ viruses were resistant to 10 μM RBV (Figure 1B), while MHV-ExoN− virus titers decreased by ~200-fold following treatment with 10 μM RBV. The capacity of 10 μM RBV to inhibit MHV-ExoN− replication is surprising because at least 10-fold higher concentrations of RBV are required to inhibit poliovirus and chikungunya viruses [48–50]. This observation could be due to the longer genomes of CoVs or to the mechanism(s) by which RBV inhibits CoV replication.

The antiviral activity of RBV against ExoN− viruses is not primarily due to mutagenesis

If RBV is exerting antiviral activity primarily through mutagenesis following incorporation of RMP, MHV-ExoN− viruses should exhibit increased sensitivity during multi-cycle replication. To test this, we determined the sensitivity of MHV-ExoN+ and ExoN− viruses to RBV at a low multiplicity of infection (MOI = 0.01 PFU/cell). Unexpectedly, multi-cycle replication of MHV-ExoN− viruses in the presence of RBV (Figure 1B) was indistinguishable from single-cycle replication.

RBV has been reported to exert antiviral activity through numerous mechanisms [38] including disruption of viral RNA synthesis and inhibition of the cellular enzyme inosine monophosphate dehydrogenase (IMPDH). To determine if RBV treatment was affecting CoV RNA synthesis, we performed two-step real-time quantitative reverse transcription PCR (real-time qRT-PCR)

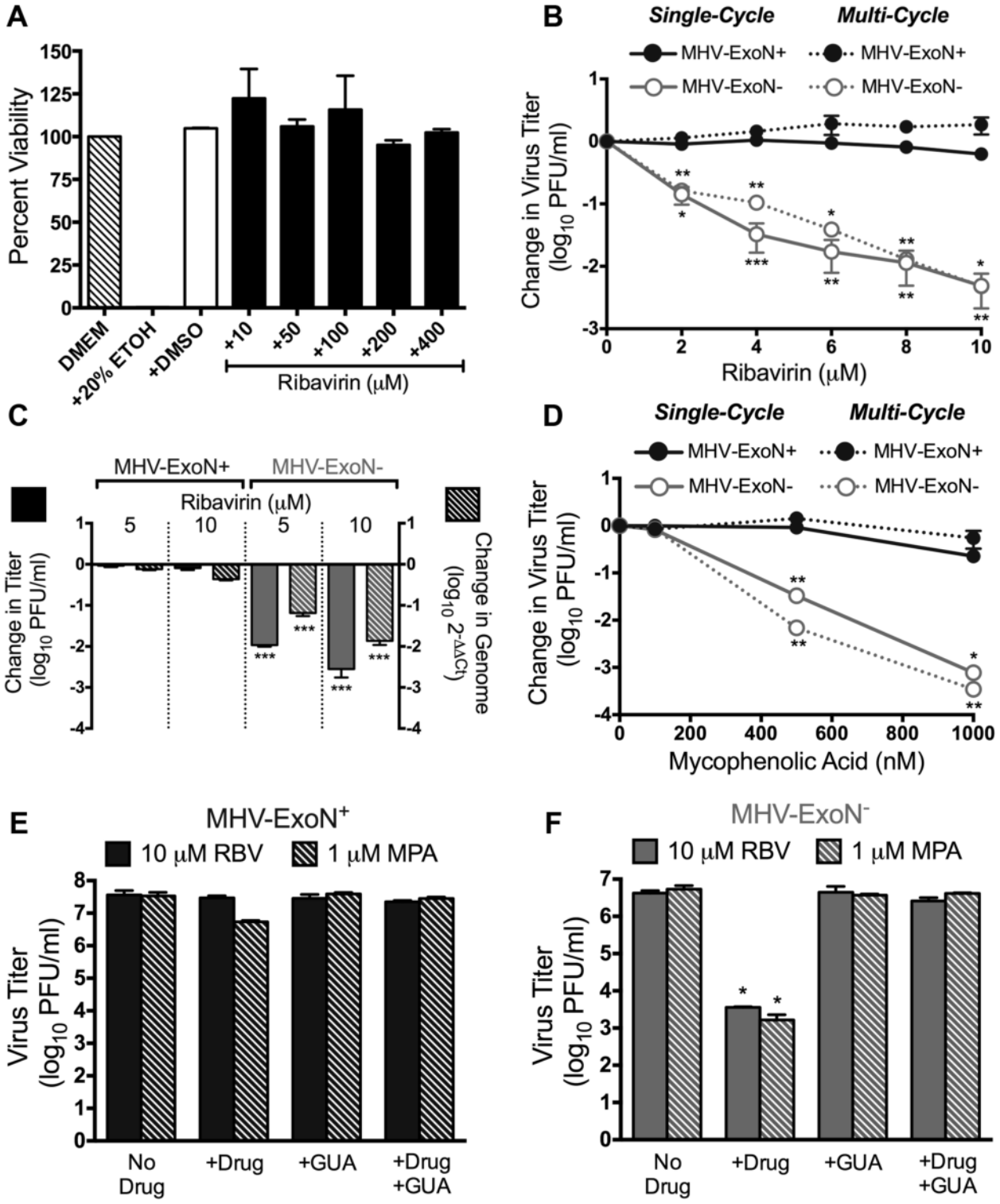

Figure 1. The antiviral activity of RBV against ExoN− viruses is not primarily due to mutagenesis. (**A**) DBT cells in 96-well plates were incubated with DMEM alone, or DMEM containing 20% ethanol (EtOH), 4% DMSO, or the indicated concentration of RBV for 12 h. Cell viability was determined using CellTiter-Glo (Promega) according to manufacturer's instructions. All values were normalized to the untreated (DMEM) control. No significant differences were found when RBV-treated values were compared to DMEM samples containing DMSO (+DMSO) using an unpaired, two-tailed Student's *t* test. Mean values ± S.E.M. are shown, n = 2. (**B**) MHV-ExoN+ (filled circle) and MHV-ExoN− (open circle) virus sensitivity to RBV during single- (solid lines; MOI = 1 PFU/cell) and multi-cycle (dotted lines; MOI = 0.01 PFU/cell) replication. MHV-ExoN+ viruses are shown in blue and MHV-ExoN− viruses are shown in green. The change in virus titer was calculated by dividing virus titers following treatment by the untreated controls. Mean values ± S.E.M. are shown, n = 4. (**C**) The change in titer (filled bars) and genomic RNA levels (hatched bars) of MHV-ExoN+ (blue) and MHV-ExoN− (green) viruses following treatment with RBV is shown. DBT cells were infected with MHV-ExoN+ or MHV-ExoN− in the presence or absence of RBV, and virus titer was determined by plaque assay. Genomic RNA levels were determined using two-step real-time qRT-PCR and primers

optimized to amplify a ~120 nt region of ORF1a [33]. The change in genomic RNA levels ($2^{-\Delta\Delta Ct}$) is shown relative to endogenous GAPDH expression and was normalized to RNA levels from untreated samples. Mean values ± S.E.M. are shown, n = 6. (**D**) MHV-ExoN+ (filled circle) and MHV-ExoN− (open circle) virus sensitivity to mycophenolic acid (MPA) during single- (solid lines; MOI = 1 PFU/cell) and multi-cycle (dotted lines; MOI = 0.01 PFU/cell) replication. Mean values ± S.E.M. are shown, n = 2–4. RBV- or MPA-treated MHV-ExoN+ (**E**) and MHV-ExoN− (**F**) viruses with or without the addition of 100 μM guanosine (GUA) during single-cycle replication (MOI = 1 PFU/cell). Mean values ± S.E.M. are shown, n = 2. For all parts, statistical significance was determined using an unpaired, two-tailed Student's *t* test (*P<0.05, **P<0.01, ***P<0.0001).
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to determine viral genomic RNA levels in the presence or absence of RBV. Similar to Figure 1B, MHV-ExoN+ titers were unaffected, whereas there was a dose-dependent reduction in MHV-ExoN− titers following RBV treatment (Figure 1C, filled bars). Corresponding dose-dependent reductions in MHV-ExoN− genomic RNA were observed (Figure 1C, hatched bars) following RBV treatment, demonstrating that treatment with 10 μM RBV decreased MHV-ExoN− RNA synthesis by nearly 100-fold during replication. Because RBV caused decreased RNA synthesis in MHV-ExoN− viruses, we calculated the relative specific infectivities of both viruses at each RBV concentration (Table 1). The relative specific infectivity of MHV-ExoN− viruses was decreased by 6- to 9-fold following treatment with RBV, while MHV-ExoN+ viruses were unaffected.

In addition to decreasing viral RNA synthesis, RBV could be exerting antiviral activity against MHV-ExoN− through competitive inhibition of IMPDH by RMP [51]. To test this possible mechanism, we treated MHV-ExoN+ and MHV-ExoN− viruses with the specific IMPDH inhibitor mycophenolic acid (MPA; [52–54]) during both single- and multi-cycle replication. A concentration-dependent decrease in MHV-ExoN− virus titer was observed following MPA treatment during single-cycle replication (Figure 1D). MHV-ExoN+ titers were reduced by less than 10-fold, consistent with what was observed following RBV treatment (Figure 1B). Similar to RBV, increased sensitivity of MHV-ExoN− viruses to MPA was not observed during multi-cycle replication. If RBV is acting via IMDPH inhibition, addition of extracellular guanosine (GUA) should restore virus titers, as has been demonstrated previously for Dengue virus [55]. Addition of 100 μM GUA following RBV or MPA pretreatment and viral infection had no effect on MHV-ExoN+ viruses (Figure 1E), but completely restored MHV-ExoN− titer even in the continued presence of 10 μM RBV or 1 μM MPA (Figure 1F). These data indicate that the antiviral activity of RBV against MHV-ExoN− viruses is occurring at least in part through decreasing viral RNA synthesis and inhibition of IMPDH. Because our primary goal was to test the role of nsp14-ExoN in the prevention and/or removal of nucleotide misincorporation we did not further investigate how RBV was specifically inhibiting ExoN− viruses. However, these results do show that the presence of ExoN activity is capable of preventing RBV inhibition of CoV replication.

The increased sensitivity of MHV-ExoN− viruses to 5-FU treatment is consistent with mutagenesis

We next examined the sensitivity of MHV-ExoN+ and ExoN− viruses to the pyrimidine base analog 5-FU, which has been shown to be mutagenic for many RNA viruses [29,56]. Treatment of DBT cells with up to 400 μM 5-FU did not result in any detectable cellular toxicity (Figure 2A). Following treatment with up to 200 μM 5-FU (Figure 2B) during single-cycle infections, MHV-ExoN+ titers were inhibited less than 3-fold, while titers of MHV-ExoN− decreased ~900 fold, representing a ~300-fold increase in sensitivity as compared to MHV-ExoN+. During multi-cycle replication, MHV-ExoN+ virus titers were reduced by less than 10-fold following 5-FU treatment, while MHV-ExoN− showed a ~50,000-fold reduction in titer (Figure 2B). Virus was undetectable by plaque assay at 5-FU concentrations above 80 μM. Analysis of viral RNA synthesis by two-step real-time qRT-PCR demonstrated that MHV-ExoN+ RNA levels were not reduced following 5-FU treatment, while 5-FU treatment resulted in minimal two-to-five fold decreases in MHV-ExoN− RNA (Figure 2C). The specific infectivity of MHV-ExoN− was decreased by 14- and 128-fold following treatment with 100 μM and 200 μM 5-FU, respectively (Table 1). These results demonstrate that ExoN activity confers resistance to 5-FU, and support the hypothesis that 5-FU is driving increased genomic mutagenesis in MHV-ExoN− virus populations, leading to lethal mutagenesis and extinction.

SARS-ExoN− viruses are sensitive to 5-FU treatment

To determine whether SARS-CoV viruses lacking ExoN activity (SARS-ExoN−) also were inhibited by RBV and 5-FU, we infected Vero cells with either SARS-ExoN+ or ExoN− viruses in the presence or absence of RBV or 5-FU. Treatment of Vero cells with up to 400 μM RBV or 5-FU did not decrease cell viability by more than 20% (Figure 3A). Recent reports have

Table 1. Relative specific infectivities of MHV-ExoN+ and ExoN− viruses following treatment with RBV or 5-FU.

Virus	RBV(μM)	Relative Specific Infectivity	Fold Decrease	5-FU (μM)	Relative Specific Infectivity	Fold Decrease
MHV-ExoN+	0	1		0	1	
	5	1.2±0.1	0.84±0.06	100	0.33±0.05	3.4±0.5
	10	1.9±0.2	0.56±0.05	200	0.24±0.03	4.5±0.4
MHV-ExoN−	0	1		0	1	
	5	0.19±0.04	6.0±0.7***	100	0.10±0.03	13.6±2.9**
	10	0.26±0.11	9.1±3.0*	200	0.012±0.004	128±29**

Relative specific infectivity values were calculated using the data shown in Figures 1C and 2C and represent the change in virus titer divided by the change in virus genome for each sample. All values are shown relative to untreated virus. The mean value and standard error for each sample is shown (Student's *t* test, n = 4, *P<0.05, **P<0.01, ***P<0.0001).
doi:10.1371/journal.ppat.1003565.t001

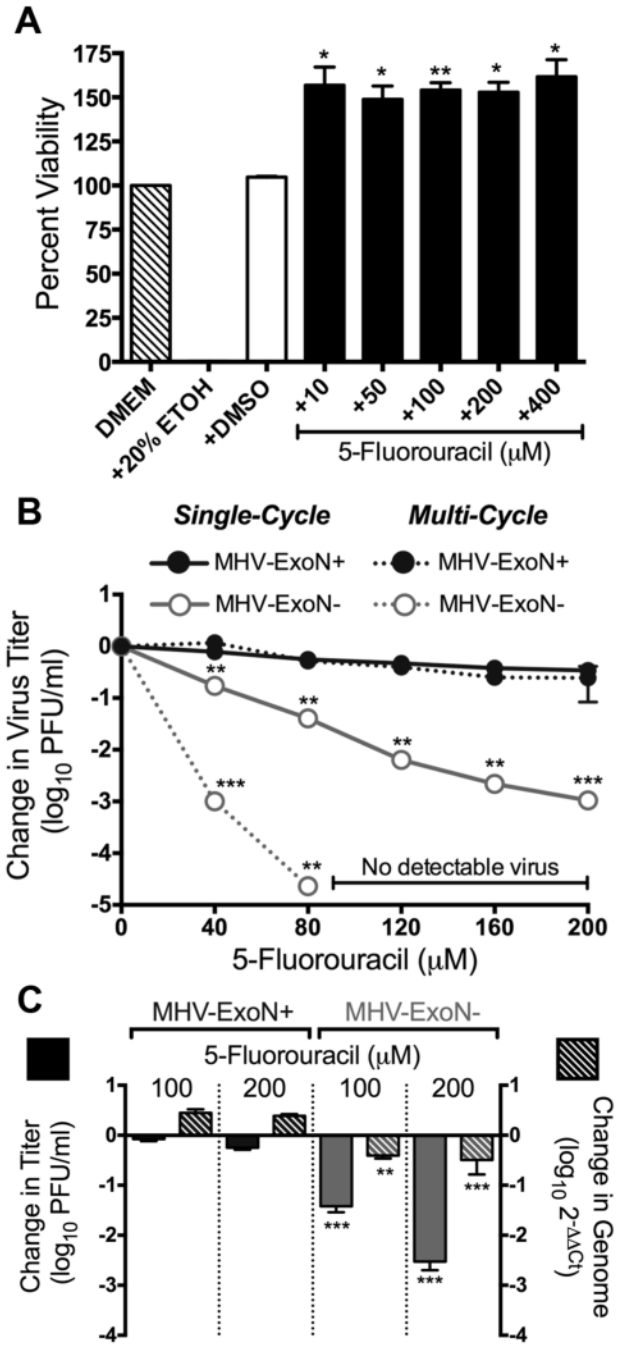

Figure 2. The increased sensitivity of MHV-ExoN− viruses to 5-FU is consistent with mutagenesis. (**A**) DBT cells in 96-well plates were incubated with DMEM alone, or DMEM containing 20% ethanol (EtOH), 4% DMSO, or the indicated concentration of 5-FU for 12 h. Cell viability was determined using CellTiter-Glo (Promega) according to manufacturer's instructions. All values were normalized to the untreated (DMEM) control. Mean values ± S.E.M. are shown, n = 2. (**B**) MHV-ExoN+ (filled circle) and MHV-ExoN− (open circle) virus sensitivity to 5-FU during single- (solid lines; MOI = 1 PFU/cell) and multi-cycle (dotted lines; MOI = 0.01 PFU/cell) replication. MHV-ExoN+ viruses are shown in blue and MHV-ExoN− viruses are shown in green. The change in virus titer was calculated by dividing virus titers following treatment by the untreated controls. Mean values ± S.E.M. are shown, n = 4. (**C**) The change in titer (filled bars) and genomic RNA levels (hatched bars) of MHV-ExoN+ (blue) and MHV-ExoN− (green) viruses following treatment with 5-FU is shown. DBT cells were infected with MHV-ExoN+ or MHV-ExoN− in the presence or absence of 5-FU, and virus titer was determined by plaque assay. Genomic RNA levels were determined using two-step real-time qRT-PCR and primers optimized to amplify a ~120 nt region of ORF1a [33]. The change in genomic RNA levels ($2^{-\Delta\Delta Ct}$) is shown relative to endogenous GAPDH expression and was normalized to RNA levels from untreated samples. Mean values ± S.E.M. are shown, n = 6. For all parts, statistical significance was determined using an unpaired, two-tailed Student's *t* test (*P<0.05, **P<0.01, ***P<0.0001).
doi:10.1371/journal.ppat.1003565.g002

described the lack of RBV uptake by Vero cells due to the absence of specific equilibrative nucleoside transporters [57,58]. Additionally, previous studies have shown that RBV failed to inhibit SARS-CoV replication in Vero cells [59]. Consistent with those reports, in our experiments both SARS-ExoN+ and ExoN− viruses were unaffected by treatment with up to 400 μM RBV (Figure 3B). We therefore performed subsequent experiments with 5-FU. SARS-ExoN+ titers were reduced 3- and 10-fold following treatment with 200 or 400 μM 5-FU, respectively (Figure 3C). In contrast, SARS-ExoN− titers were reduced ~300-fold by 200 μM 5-FU (Figure 3C), similar to MHV-ExoN− viruses. At 400 μM 5-FU, SARS-ExoN− virus was inhibited 2,000-fold during a single replication cycle, representing a ~160-fold increase in 5-FU sensitivity compared to SARS-ExoN+ viruses. Thus, our data indicate that increased sensitivity of CoVs to RNA mutagens in the absence of ExoN activity is conserved across diverse members of the CoV family. Of interest, our studies with SARS-ExoN+ also indicate that ExoN-mediated protection from nucleotide mis-incorporation can be overcome at higher concentrations of mutagen.

5-FU drives increased mutagenesis in both SARS-ExoN+ and ExoN− viruses

Studies with the RNA viruses lymphocytic choriomeningitis virus (LCMV), foot-and-mouth disease virus (FMDV) and vesicular stomatitis virus (VSV) have demonstrated that 5-FU is incorporated as 5-fluorouridine monophosphate (FUMP) into replicating viral RNA, thus increasing genomic mutations [60–62]. To determine whether 5-FU was causing increased mutagenesis in SARS-CoV populations, we performed full-genome NGS analysis of both virus populations replicating in the presence or absence of 5-FU. To analyze the entire spectrum of mutations arising during replication, we extracted total intracellular RNA from Vero cells infected with either SARS-ExoN+ or ExoN− viruses following treatment with either 0 μM or 400 μM 5-FU. We then generated 12 overlapping cDNA amplicons of approximately 3 kb in length for each sample. For each of the four samples, 1.4×10^8 to 4.5×10^8 bases were sequenced, corresponding to an average coverage depth of between 4,600 and 15,000 at each nucleotide position. We compared the statistically significant minority variants, defined as having a p-value of ≤0.05 following a multiple-testing correction (Benjamini-Hochberg), between the untreated and 5-FU-treated SARS-ExoN+ and ExoN− populations. Following treatment with 400 μM 5-FU (Figure 3D), there was an increase in mutations within the SARS-ExoN+ population from 11 to 259 (24-fold). In contrast, for SARS-ExoN− there were 3648 mutations present within the 5-FU-treated SARS-ExoN− population compared to the 99 mutations in the untreated population (40-fold increase). Most remarkably, this represented a 16-fold increase in the number of statistically significant minority variants between 5-FU treated ExoN+ and ExoN− SARS-CoV. Thus, these data support our hypothesis that 5-FU was increasing genomic mutations through incorporation of FUMP into viral genomes in the absence of ExoN activity.

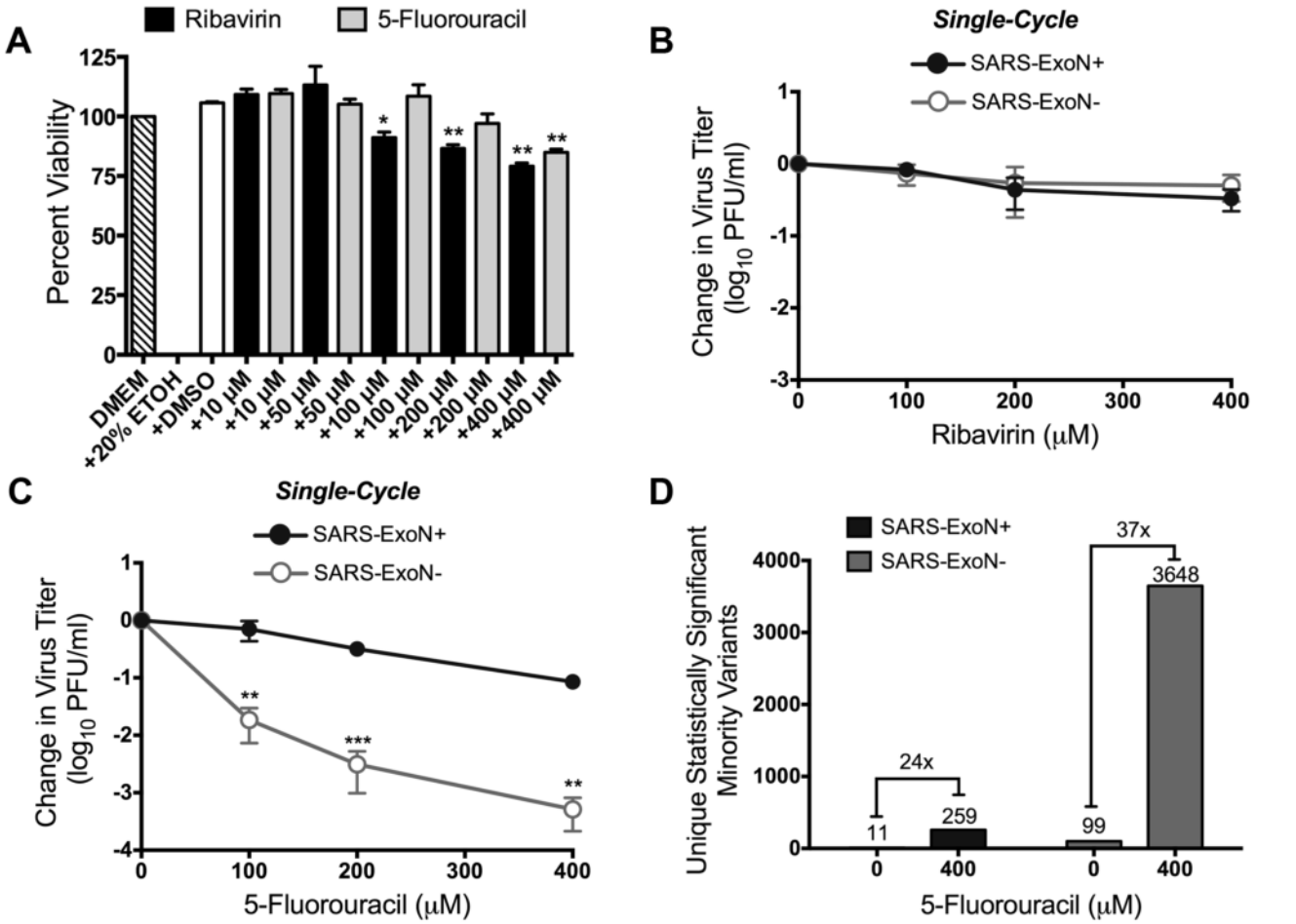

Figure 3. SARS-ExoN− viruses have increased sensitivity to 5-FU. (**A**) Vero cells in 96-well plates were incubated with DMEM alone, or DMEM containing 20% ethanol (EtOH), 4% DMSO, or the indicated concentration of RBV or 5-FU for 24 h. Cell viability was determined using CellTiter-Glo (Promega) according to manufacturer's instructions. All values were normalized to the untreated (DMEM) control. Mean values ± S.E.M. are shown, n = 3. The change in SARS-ExoN+ (filled blue circles) and SARS-ExoN− (empty green circles) titers following treatment with RBV (**B**) or 5-FU (**C**) during single-cycle replication. Vero cells were infected with either virus at an MOI of 0.1 PFU/cell, and virus supernatant was harvest 24 h post-infection following replication in the presence or absence of RBV or 5-FU. Virus titer was determined by plaque assay on Vero cells. Mean values ± S.E.M. are shown, n = 2 (RBV) and n = 4 (5-FU). (**D**) Comparison of unique statistically significant ($P<0.05$) minority variants present between untreated and 5-FU treated samples for both SARS-ExoN+ and ExoN− populations. SARS-ExoN+ viruses are shown in blue, and SARS-ExoN− viruses are shown in green. For panels A–C statistical significance was determined using an unpaired, two-tailed Student's *t* test (*$P<0.05$, **$P<0.01$, ***$P<0.0001$).
doi:10.1371/journal.ppat.1003565.g003

5-FU-associated A-to-G and U-to-C transitions are highly represented and distributed across the genome

Incorporation of FUMP instead of uracil into replicating RNA allows FUMP to base pair with both guanosine and adenine [61,63]. This decreased specificity in base pairing has been shown in studies with LCMV and primarily results in A-to-G (A:G) and U-to-C (U:C) transitions [29,61,63]. To determine if FUMP was being incorporated at higher levels in the absence of ExoN-mediated proofreading, we analyzed the numbers and types of transitions and transversions occurring in each virus population (Figure 4). Transitions are indicated in grey boxes and transversions in white boxes, with the number for each shown. Transversions comprised the majority of variants for both untreated ExoN− and ExoN+ viruses. Treatment with 5-FU caused the number of U:C and A:G transitions to increase in both ExoN+ and ExoN− populations, from 2 to 197 for SARS-ExoN+ and from 16 to 3304 for SARS-ExoN− (Figures 4A and B). This increase and bias toward U:C and A:G transitions is consistent with FUMP being incorporated into both minus- and plus-strand RNA [63] during both ExoN+ and ExoN− replication; however the absolute numbers were dramatically increased (16-fold) during ExoN− replication compared to ExoN+. In untreated cells, A:G and U:C transitions accounted for less than 25% of the total minority variants within each population (Figure 4C). Following 5-FU treatment, A:G and U:C transitions accounted for 70–95% of the total minority variants within each population.

To further examine the genomic distribution of these two transitions, we plotted the total number of A:G and U:C transitions occurring at a frequency of between 0.1% and 1% (Figure 5). Approximately 75% and 90% of the total minority variants occurring at a frequency between 0.1 and 1% following 5-FU treatment were due to A:G or U:C transitions (Figure 5), for the SARS-ExoN+ and ExoN− populations, respectively. In both populations, these mutations were distributed across the entire genome following treatment with 400 μM 5-FU. Thus our data provide direct evidence indicating that 5-FU drives increased genomic mutations within SARS-CoV in the absence of ExoN proofreading activity.

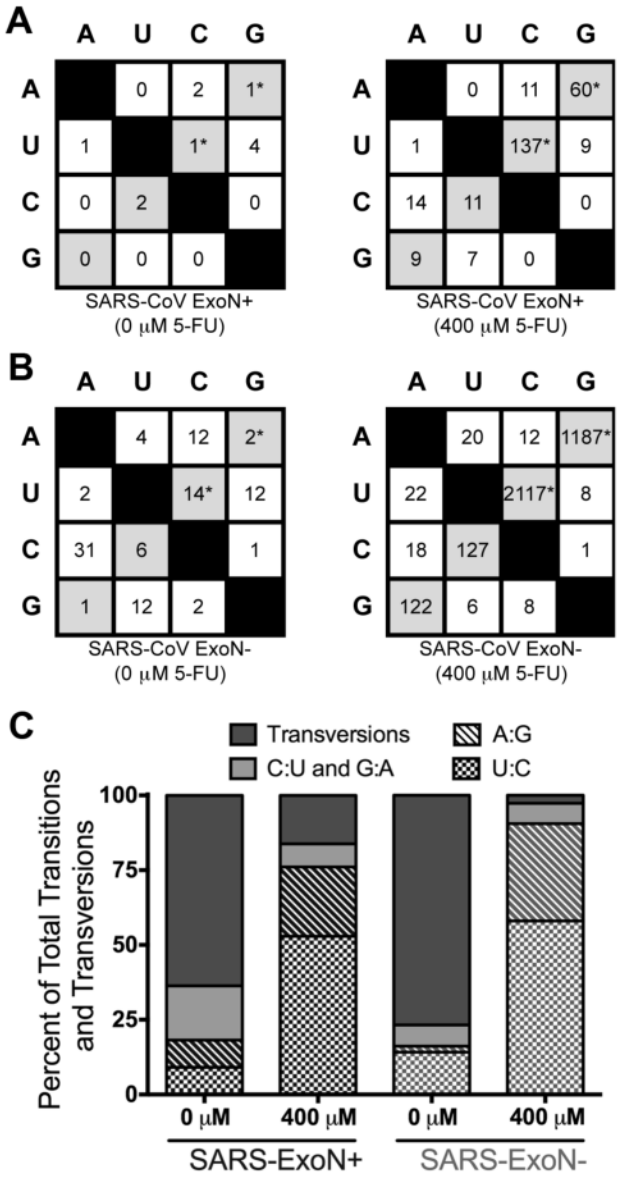

Figure 4. Incorporation of FUMP results in increased U:C and A:G transitions. All possible base changes are shown for SARS-ExoN+ and SARS-ExoN− viruses in panels (**A**) and (**B**), respectively. Transitions (A↔G and U↔C) are shaded in grey, and 5-FU specific transitions (U:C and A:G) are marked with an asterisk. Transversions (A↔T, A↔C, C↔G, G↔T) are shown in white boxes. All values represent the number of unique statistically significant minority variants following 5-FU treatment. (**C**) The percent of all unique statistically significant minority variants represented by transversions (filled dark grey bars), C:U and G:A transitions (filled light grey bars), and the 5-FU specific transitions A:G (hatched bars) and U:C (checkered bars) are shown following 0 or 400 μM 5-FU treatment. SARS-ExoN+ viruses are shown in blue, and SARS-ExoN− viruses are shown in green.
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Discussion

Viral sensitivity to RNA mutagens is determined by several factors including polymerase selectivity [39,40,64–67], mutational robustness [68], and the acquisition of mutations that increase or decrease replication fidelity. Increased and decreased fidelity mutants have been described for picornaviruses and arboviruses [35,48,50,69], all of which have occurred in the viral RdRp. The CoV nsp14-ExoN is the first identified RNA virus protein distinct from the RdRp that affects replication fidelity [19–21,70]. While the G641D mutation within the chikungunya (CHIKV) nonstructural protein 2 (nsP2) has been implicated in CHIKV resistance to RBV, a direct role for this protein in fidelity regulation has not been described [48]. A Sindbis virus variant containing mutations within nsP1, a viral guanylyl- and methyltransferase [71], has been shown to be resistant to both RBV and MPA [72]. However, this phenotype is related to viral RNA capping and not replication fidelity [72]. In this report, we identify CoV ExoN activity as a critical determinant of viral sensitivity to RNA mutagens. Using two phylogenetically distant β-CoVs we demonstrate that this phenotype is well conserved across CoVs. Clearly, there is a profound increase both in overall mutations and in specific 5-FU-associated mutations within the ExoN− population as compared to the ExoN+ wild-type population. Furthermore, the vast majority of statistically significant mutations were distributed genome-wide at frequencies between 0.2 and 1%, providing strong evidence supporting ExoN-mediated proofreading during CoV replication. Of interest, our experiments also revealed that ExoN-mediated prevention and/or removal of misincorporated nucleotides is not absolute, especially in the setting of higher concentrations of mutagen. ExoN+ SARS-CoV populations demonstrated 24-fold more mutations following 5-FU treatment, suggesting that ExoN proofreading can be overwhelmed by higher concentrations of mutagens and likely by other nucleoside or base analogs. This raises the further possibility that ExoN may be less efficient at recognizing or removing some types of nucleoside or base analogs than others, and that such approaches to virus inhibition might be viable, particularly in combination with inhibitors that target ExoN activity.

Ribavirin activity against CoVs is not primarily due to mutagenesis

The antiviral nucleoside analog RBV is currently used to treat hepatitis C virus (HCV; [73–75]), Lassa virus [76] and respiratory syncytial virus (RSV) infections [77,78]. The potential clinical use of RBV for CoV infections is complicated by the multiple mechanisms of action that have been reported [38], and by the potential for disease exacerbation, as reported during the SARS-CoV epidemic [25–28]. Our data suggest that RBV primarily inhibits MHV-ExoN− virus replication through decreasing viral RNA synthesis and inhibition of IMPDH (Figure 1). Inhibition of IMPDH by RMP has been shown to decrease intracellular GTP pools [51], thus altering the balance of nucleoside triphosphates (NTPs) within the cell. Decreased GTP levels could result in forced misincorporations due to NTP imbalances in the absence of ExoN activity [72]. However, the moderate 6- to 9-fold decreases in relative specific infectivity observed for MHV-ExoN− following RBV treatment (Table 1) suggests that mutagenesis is not the primary mechanism by which RBV is exerting an antiviral effect. An additional possibility is that the antiviral activity of RBV against ExoN− viruses is unrelated to the putative proofreading function of this enzyme. Both biochemical and cell culture studies have demonstrated that loss of ExoN activity leads to impaired RNA synthesis [15,19,20]. Furthermore, in addition to ExoN activity, nsp14 contains N7-methyltransferase (N7-MTase) activity, a critical step in RNA capping [79,80]. A recent report has demonstrated that the ExoN and N7-MTase domains are structurally inseparable, and that residues within the ExoN domain are important for N7-MTase activity [81]. Thus, the increased sensitivity of MHV-ExoN− to RBV could result from the impairment of undefined functions of ExoN during CoV replication, particularly during RNA synthesis. The parallel use of

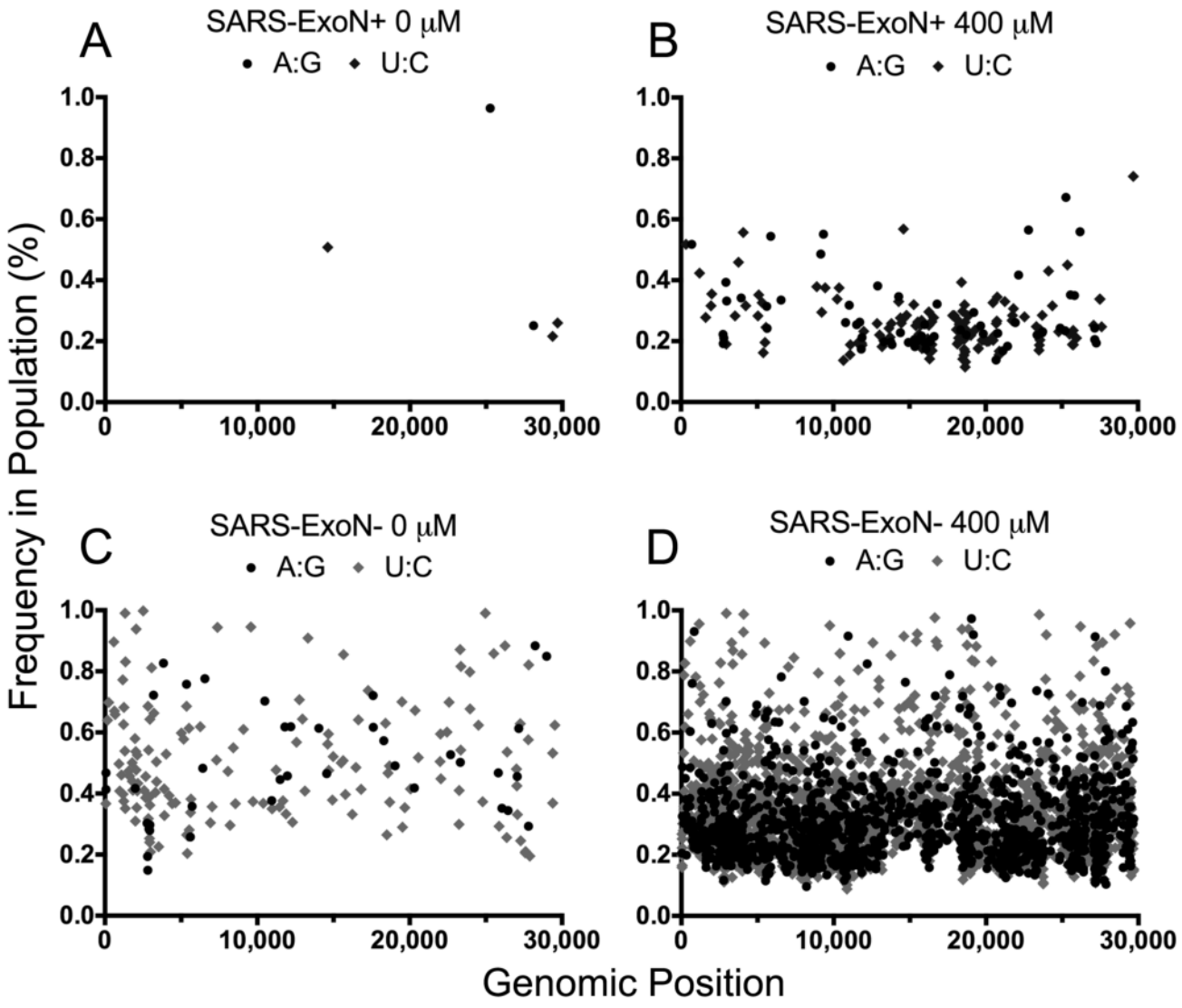

Figure 5. 5-FU-mediated U:C and A:G transitions are distributed across the CoV genome at low frequency. (**A**) and (**B**) The genomic distribution of low frequency statistically significant U:C and A:G variants within the SARS-ExoN+ population following treatment with 0 or 400 μM 5-FU. (**C**) and (**D**) Same as in A and B except for the SARS-ExoN− population. For all panels, SARS-ExoN+ viruses are shown in blue, and SARS-ExoN− viruses are shown in green. U:C transitions are denoted by a diamond, whereas A:G transitions are plotted as circles.
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ExoN+ and ExoN− viruses with RBV may allow us to define how RBV is exerting an antiviral effect against CoVs and the potentially novel mechanisms by which ExoN may act to counter that inhibition.

ExoN proofreading during CoV replication

Since the identification of nsp14-ExoN activity [15] and studies demonstrating the requirement for ExoN in high-fidelity replication [19–21], mounting evidence points to a role for nsp14-ExoN in proofreading activity during RNA virus replication [22]. Here we used NGS to determine the number of mutations present in SARS-ExoN+ and ExoN− populations. The characteristic 5-FU-mediated transitions U:C and A:G comprised 90% of the total statistically significant minority variants within SARS-ExoN− population, and were present at levels 15- and 20-fold higher than those same transitions within the ExoN+ population (Figure 4). Overall, our data represent the first direct test of ExoN proofreading during SARS-CoV replication in the absence of ExoN. Furthermore, the sequencing depth attained using NGS shows that ExoN inactivation likely skews the spectrum of spontaneous mutations present within the untreated population (Figure 4). Such overrepresentation of specific mutations in the context of ExoN inactivation is similar to studies of *S. cerevisiae* DNA polymerases ε and δ containing mutations within their respective 3′-to-5′ DEDD exonucleases [82–86]. This altered distribution due to ExoN inactivation could have profound implications for CoV adaptation and evolution.

Nsp14-ExoN as a target for combination CoV inhibitors

Lethal mutagenesis occurs through the accumulation of mutations within the viral genome during replication, and ultimately results in virus extinction (reviewed in [56,87]). While lethal mutagenesis has been studied extensively [87], our work is the first to identify an RNA virus protein distinct from the RdRp that directly regulates the sensitivity of RNA viruses to genomic mutations resulting from mutagen incorporation. Currently, RBV

is the only FDA-approved antiviral with demonstrated mutagenic activity. The first demonstration of RBV acting as a mutagen was performed using poliovirus [23,24] almost 30 years after the antiviral activity of RBV was described [88]. The nucleoside analog T-705 (Favipiravir; [89]) is currently in clinical development, and has been shown recently to drive lethal mutagenesis of influenza virus [90]. We have shown that ExoN+ viruses replicate well in the presence of RBV or 5-FU. However, we also have shown that ExoN− mutants of SARS-CoV and MHV have 15-to-20-fold decreased fidelity [19,20], are attenuated, are subject to rapid loss of replication and clearance *in vivo* [21], and are highly susceptible to low concentrations of RNA mutagens. An exciting possibility is that this conserved CoV proofreading enzyme could be targeted for inhibition, thus leading to the development of broadly useful CoV therapeutics. While ExoN inhibitors alone might be efficacious, combining an inhibitor of CoV fidelity with an RNA mutagen would magnify the intrinsic fidelity defect of ExoN inhibition and drive high-level mutagenesis. A potential advantage of such an approach would be to rapidly drive the virus to extinction, while limiting or blocking the capacity of the virus to overcome inhibition by reversion. ExoN− mutants of both MHV and SARS-CoV have shown no reversion over multiple passages in culture or during persistent infections *in vivo* [19–21]. Furthermore, we did not observe any primary reversions within the ExoN DEDD motif following 5-FU treatment. While mutations within the CoV RdRp could emerge during acute treatment, mutations within other RNA virus RdRps have demonstrated that the maximum tolerance for increased or decreased fidelity without loss of virus viability is between ~3- to 6-fold [35,48,69,91]. In addition, our data demonstrate that ExoN− viruses are profoundly sensitive to inhibition by lower concentrations of mutagen, providing a possible improved therapeutic index and margin of safety for use.

In summary, this study provides the most direct evidence to date that CoV ExoN provides a proofreading function during virus replication, and identifies ExoN as the critical determinant of CoV sensitivity to RNA mutagens. Because CoV replication fidelity is likely determined by the concerted effort of multiple virus proteins [19,20,22], our data suggest the exciting possibility that significant attenuation of CoV fitness and pathogenesis could be achieved by targeting the conserved process of CoV replication fidelity. Ultimately, uncovering the mechanism of fidelity regulation and methodologies to disrupt this critical process will be vital to responding to both endemic and future emerging CoVs such as SARS-CoV and MERS-CoV.

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Author Contributions

Conceived and designed the experiments: ECS MV MRD. Performed the experiments: ECS. Analyzed the data: ECS HB. Wrote the paper: ECS MV MRD.

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From: Denison, Mark (NIH)
Sent: Tue, 21 Oct 2014 19:21:26 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Baric, Ralph
Cc: (b)(6)
Subject: Re: AI108197- No GOF

]Erik,

Will do on the Business office and I will have the summary comments to you in the next hour.
Also I forwarded to you my responses to Maureen Beanan about the U19 aims. Together with the R01 that represents all of the funded work on SARS and MERS that might be relevant.

I will forward my emails through our business office so there can be a formal response there as well.
Thanks for quick responses and call.

Regards

Mark

From: <Stemmy>, "Erik [E] (NIH/NIAID)" (b)(6)
Date: Tuesday, October 21, 2014 2:09 PM
To: Mark Denison (b)(6)
Subject: RE: AI108197- No GOF

Hi Mark,
Thanks for taking the time to chat this morning, it definitely cleared up our questions about the GoF potential of Aim 3 of your grant. I just wanted to let you know that your business office will still likely receive a message from grants management about it. As we discussed you can put together a brief paragraph summarizing the additional data and that there's no reasonable assumption of increased pathogenesis and then reply through your business office as well.

Thanks again for being so willing to engage us over this topic!

Best,
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Denison, Mark (NIH)
Sent: Monday, October 20, 2014 2:20 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: AI108197- No GOF

9:30 eastern? Yes. 8:30 central.

Sent from my iPhone

On Oct 20, 2014, at 1:18 PM, "Stemmy, Erik (NIH/NIAID) [E]" (b)(6) wrote:

Hi Mark,
Thanks very much for sending the summary. Would you be available for a short call tomorrow morning at 9:30 to discuss? Please let me know.

Thanks!
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Denison, Mark (NIH)
Sent: Monday, October 20, 2014 12:02 PM
To: Stemmy, Erik (NIH/NIAID) [E]; Spiro, David (NIH/NIAID) [E]
Cc: (b)(6) Baric, Ralph; Sims, Amy C
Subject: AI108197- No GOF

Dear Erik and David,

I expect you are not surprised to hear from us!
I (Mark) would be happy to talk if you think we should. I can always be reached by email or call my phone (b)(6) (text also if emergent)

Just an update. These are the current aims of **AI 108197 Determinants of replication fidelity. (Denison, Baric). NO GOF studies**

We are making great progress. As below, most of initial mutagenesis and testing for proof of concept is in MHV, then SARS and MERS
Importantly, all data demonstrates that decreased fidelity is attenuating in SARS animal model, and increased fidelity is less fit in vitro (soon to be submitted)

We are doing passage with MERS (in vitro) to allow enough replication to recover the ExoN- viruses. We are using the MERS-CoV clone from Ralph. Stored as fragments, all work at BSL3.

Note in **Aim 3 part 3 we do test for reversion to virulence** – to understand the stability of the Exon- genotype and phenotype during in vivo infection. This is not GOF, but rather testing for restoration of virulence after attenuation by ExoN or other mutations.

Let me know if you have any questions or need more details about our work. Of course I will provide any detailed information you need

Aim 1. To define nsp14 fidelity determinants and their impact on SARS-CoV and MERS-CoV replication and fitness. In **part 1,** we will use MHV and SARS-CoV to test the effect of predicted and systematic mutations in nsp14-ExoN motifs and residues, Zn finger domain, conditional (ts) alleles, conserved charged residues outside of the ExoN motif, and the carboxy-terminal N7-methyltransferase domain in nsp14 on replication fidelity by next generation sequencing and mutagen sensitivity. Experiments in **part 2** will test the impact of altered fidelity on virus genotypic and phenotypic stability and competitive fitness during infection and passage in culture. In **part 3** we will use the newly established reverse genetic system for **MERS-CoV** to test for conservation of ExoN mediated fidelity and fidelity altering mutations on replication in multiple continuous and primary cell lines of the human lung.

Aim 2. To define the effect of nsp14-ExoN fidelity altering mutations on RNA synthesis, and on exonuclease and N7-methyltransferase activity in vitro. In **part 1** we will determine the effect of increased and decreased fidelity mutations on RNA synthesis and recombination **for SARS-CoV and MERS-**CoV. In **part 2,** we will determine the in vitro biochemical mechanism of activity of altered fidelity mutations in vitro on nsp14 Exonuclease and N7-methyltransferase activity. In **part 3** we will determine the sensitivity of nsp14 mutants to RNA mutagens, nucleoside analogs and β-IFN, testing the mechanism action during infection.

Aim 3. To determine the effect of altered fidelity on *in vivo* replication and pathogenesis. We will test the *hypothesis that decreased or increased fidelity is attenuating for SARS-CoV and MERS-CoV replication and pathogenesis in vivo, while allowing protective immune response*. In **part 1** we will use selected increased and decreased fidelity mutants to test replication and pathogenesis in aged, immunocompromised and persistently infected mice of different genetic backgrounds. In **part 2,** we will determine minimal lethal dose, lung pathology, tissue tropism and effects on respiratory function in young and aged mice, in order to define the limits of fidelity regulation on in vivo pathogenesis in the lung. **In part 3,** we will test increased and decreased fidelity mutants during in vivo passage for genotypic and phenotypic stability and reversion to virulence. In **part 4** we will apply results from **parts 1-3** in animal models of **MERS-CoV** to test conserved attenuating ExoN fidelity mutants on replication, pathogenesis, immune response and stability.

Regards

Mark

Mark R. Denison M.D.

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From: Beanan, Maureen (NIH/NIAID) [E]
Sent: Tue, 21 Oct 2014 13:14:37 -0400
To: Denison, Mark (NIH); (b)(6)
Cc: Baric, Ralph; (b)(6) Sims, Amy C; (b)(6) (b)(6) Stemmy, Erik (NIH/NIAID) [E]; Schaefer, Michael (NIH/NIAID) [E]; Parker, Tina (NIH/NIAID) [E]
Subject: RE: U19 AI109680 Coronavirus Aims (Denison/Baric) regarding GOF pause

Dear Dr. Denison,
Thank you for your email. We will review this information and follow-up with you.
Best regards,
Maureen

Maureen J. Beanan, PhD
Program Officer
Office of Biodefense, Research Resources, and Translational Research
DMID/NIAID, NIH
5601 Fishers Lane
Rm. 8G28, MSC 9825
Rockville, MD 20852-9825
Phone: (b)(6)

From: Denison, Mark (NIH)
Sent: Tuesday, October 21, 2014 12:05 PM
To: Beanan, Maureen (NIH/NIAID) [E]; (b)(6)
Cc: Baric, Ralph; (b)(6) Sims, Amy C; (b)(6) Stemmy, Erik (NIH/NIAID) [E]
Subject: U19 AI109680 Coronavirus Aims (Denison/Baric) regarding GOF pause

Dear Dr. Beanan,

I am trying to be sure to communicate with program around our **Coronavirus Aims and experiments** in any proposal to allow full disclosure in regards to recent announcements about pauses in GOF studies of SARS and MERS-CoV. I just finished a conversation with Erik Stemmy about our R01 AI108197. In the interest of very clear communication and to be most helpful to Program in this time of uncertainty, I am copying this to Erik Stemmy, and will copy to you my followup email to Erik in response to our conversation.

I am co-PI along with Ralph Baric of project 2 in U19 AI109680 . (Whitley P.I.) I wanted to review the Aims with you and potential areas where you might want more information

We (Ralph and I) would be happy to talk if you think we should. I can always be reached by email (b)(6) or cell phone (b)(6)

We propose in our project to test and screen for inhibitors of Coronavirus replication (particularly against the nsp14 Exonuclease, and nsp16 methyltransferase) using MHV as a BSL2 model, screening with SARS-CoV and confirming with MERS-CoV. **** In my review of our aims, there are NO GOF studies according to the announcements that would meet criteria for "Pause" in experiments or funding.** But here are the aims, with a few comments of explanation (in blue)

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication. We will test the *hypothesis that inhibitors of CoV high-fidelity replication will decrease viral fitness alone and in combination with RNA mutagens, and represent potent pan-CoV therapeutics.* In **part 1**, we will identify ribonucleoside analogs that inhibit CoV replication, and define their mechanism of action. High-throughput screening in **part 2** will identify small-molecule inhibitors of CoV fidelity. In **part 3** we will identify the viral protein targets of lead compounds, and determine their mechanism of fidelity impairment. In **part 4**, will we test highly efficacious compounds identified in parts 1 and 2 across the CoV family and viral platforms within this program.

Comments:

- We have data identifying candidate inhibitors (nucleoside analogs) from collaboration with (b)(4); (b)(6) We are defining EC50 and will move these into studies of inhibition in animals. These are subject of active studies
- Passage (MHV model in vitro only) will identify determinants in polymerase or other proteins that interact with inhibitors
- Viruses with resistance to nucleoside analogs will be sequined to identify responsible mutations and proteins, allowing testing of activity and mechanism
- Stability of inhibition will be determinant of studies in animals.
- ****to date in our studies and others, resistance to inhibitors in vitro yields a virus LESS FIT than parent virus.**
- *****No studies are planned or performed to test for increased virulence, pathogenesis, transmissibility of host Range. EG NO GOF studies**

Aim 2. To identify and develop inhibitors of CoV RNA capping activity. We *hypothesize that small molecule inhibitors of essential CoV RNA capping components will profoundly increase CoV sensitivity to the host innate immune response through interferon-stimulated effectors.* In **part 1** we will use targeted mutagenesis of known CoV capping components to define distinct mechanisms to increase CoV sensitivity to the host ISGs. In **part 2** we will examine the combined efficacy of known OMTase inhibitors and type I IFN treatment against SARS-CoV, and perform a high-throughput screen for inhibitors of CoV RNA capping. In **part 3** we will identify the viral protein targets and mechanism of action of lead compounds. In **part 4**, lead compounds will be tested across the CoV family and specific viral platforms within this program.

Comments:

- Published by Ralphs Lab: nsp16 OMT mutants are less fit and significantly attenuated in animals
- All studies here are screening and testing for increased sensitivity to IFN and ISGs

- ****Testing in SARS and MERS will be using compounds known to inhibit replication via nsp16 or capping in vitro**
- *****No studies are planned or performed to test for increased virulence, pathogenesis, transmissibility of host Range. EG NO GOF studies**

Aim 3. To chemically optimize and test the *in vivo* efficacy of CoV fidelity and RNA capping inhibitors. We will test the *hypothesis that inhibitors of CoV fidelity or RNA capping are highly attenuating in vivo and represent broadly effective CoV therapeutics.* Compounds identified in Aims 1 and 2 will be chemically optimized for *in vitro* efficacy, selectivity, solubility, microsomal stability, and bioavailability at SR. Using these optimized compounds, in **part 1** we will confirm the biological target(s) of lead fidelity and RNA capping inhibitors *in vivo*. In **part 2** we will test the efficacy of lead compounds against mouse-adapted SARS-CoV in progressively stringent mouse models of acute and persistent human disease. Efficacy will be determined by monitoring respiratory function, morbidity and mortality, histology, and viral replication. In **part 3** we will test for the development of drug resistance *in vivo*, and will determine the efficacy of lead compounds against the novel human CoV HCoV-EMC and other CoV family members.

Comments:

- Published by Ralphs Lab: nsp16 OMT mutants are less fit and significantly attenuated in animals
- Studies are proposed to improve candidates for bioavailbility, activity and stability.
- ****Animal testing will be to pursue these aims**
- *****No studies are planned or performed to test for increased virulence, pathogenesis, transmissibility of host Range. EG NO GOF studies**

Please let me know if you need additional clarification. I am happy to share any in vitro data we have generated, based on the presentation we will give on Thursday at the CETR meeting.
Also happy to talk at any time.

Best Regards

Mark Denison

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From: (b)(6)
Date: Tuesday, October 21, 2014 9:50 AM
To: Maureen Beanan (b)(6)
Cc: Mark Denison (b)(6)
Subject: Restricted Agents

Hi Maureen,

I am copying Mark on this email. He is in the middle of the restricted agent politics and that influences his grant. I told him that he should talk to you. We meet this week.

Thanks.

(b)(6)

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thu, 19 Jun 2014 14:55:27 +0000
To: Denison, Mark (NIH)
Cc: Baric, Ralph
Subject: RE: RO1 AI108197 and possible PO1 concept

Hi Mark,
It was nice speaking with you earlier this week. I've checked in with the Grants Management folks, and they suggested contacting the eRA Commons help desk to inquire about making sure your revised aims are reflected in RePORTER. I'll paste their contact information below for you. Let me know if you have any trouble.

Best,
Erik

eRA Commons Help Desk

- **Hours:** Mon-Fri, 7AM-8PM EDT/EST
- **Web:** http://era.nih.gov/help/
- **Toll-free:** 866-504-9552
- **Phone:** 301-402-7469
- **TTY:** 301-451-5939

From: Denison, Mark (NIH)
Sent: Monday, June 16, 2014 11:17 AM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph
Subject: RO1 AI108197 and possible PO1 concept

Erik, it was good to talk with you this morning.

1) Based on our conversation, we will move forward with the modified summary / Aims / Scope of work for AI108197
Just to summarize:

- During discussions spring of 2013 on possible select pay and budget, there was a proposed reduction in budget of 20-40% due to fund availability, not based on concerns of project
- We modified budget, but also modified scope of work and Aims to match the proposed level of support (May 2013)
- We were unaware, until we received the NOGA, that the support term was reduced from 5 to 4 years. This was not considered in our modified scope of work and Aims
- However, we feel confident we can still achieve the objectives as described in the modified scope of work and Aims
- The modified aims and scope are stated in the recently submitted RPRR and will be used going forward.

2) Thanks also for conversation about possible U19/ P01 on Fidelity as the basis for broadly applicable vaccine strategies for emerging viruses. I understand probably not a lot of general support, but Thanks for being willing to look at a white paper concept. I will work with Ralph this next week to put together a summary and ideas. I appreciate the input. I also will get you our recent publications for the grant and supporting this concept.

Best Regards

Mark

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From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thu, 12 Jun 2014 14:49:18 +0000
To: Denison, Mark (NIH)
Cc: Baric, Ralph; (b)(6)
Subject: RE: Greetings and Question about AI108197

Hi Mark,
Thanks for your note. Apologies for my delayed response. We were hosting a flu meeting on Monday and Tuesday of this week and I was occupied with the last minute preparations. Let's set up a time for a quick chat so I can be sure we're on the same page. Do you have any time on Monday 6/16? I'm pretty flexible outside of the 1-2pm window. Please let me know.

Thanks!
Erik

Please note my updated contact information below:

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Denison, Mark (NIH)
Sent: Friday, June 06, 2014 4:13 PM
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; (b)(6)
Subject: Greetings and Question about AI108197

Dear Erik

We haven't had a chance to chat much this past year, but we (my lab and Ralph Barics) have made great progress on our joint PI grant AI 108197 (Determinants of Coronavirus Fidelity in

Replication and Pathogenesis). Its time for the RPRR and I wanted to be sure to touch base with you.

I am just returning home from the Coronavirus (nidovirus) meeting in Spain, so still in transit in a hotel in Madrid. Thus cant talk in person until Monday.

I wanted to review something that was completed prior to your appointment as program officer for this grant, but which I don't see reflected on the grant page. It is based on the appended documents and the email (See below) we submitted last May 14 prior to the awarding of the grant in August. It is modified Aims and scope of work based on the reduced annual award and reduction from 5 to 4 years total.

I am basing our RPRR and ongoing plans on these modified AIMs and scope of work, which as you can see from the progress report and papers, is going very well with high impact publications and progress. I just wanted to be sure these get either updated on the commons site. If we need to have any conversation I am happy to do so.
I will be available Monday June 9 and would be happy to talk with you about it.

Thanks again, and I hope to get the chance to meet you in person soon

Best Regards

Mark

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From: <Denison>, Mark Denison (b)(6)
Date: Tuesday, May 14, 2013 5:05 PM
To: "Salomon, Rachelle (NIH/NIAID) [E]" (b)(6)
Cc: David Spiro (b)(6) Ralph Baric (b)(6) "Grossman, Sonnie Kim (NIH/NIAID) [E]" (b)(6)
Subject: R01-AI-108197 Modified Aims and Response to Reviews

Dear Rachelle,

I am pleased to provide our modified Aims and Detailed Response to Reviews. I worked closely with Dr. Baric review the proposed budget modification and scope of work. We have modified the proposal to focus on the most high impact, timely and feasible studies achievable with the modified budget. In addition, our modifications are responsive to the comments in the reviews from VIRB study section. We believe that this will allow us to rapidly provide important new basic and translational discoveries applicable to both SARS-CoV and the emerging MERS-CoV.

Rachelle, I am copying to David and Sonnie as I am not sure whether you are back in the office.
Thanks to all of you for your good communication and help.

Best Regards

Mark

Mark R. Denison M.D.
Craig-Weaver Professor of Pediatrics
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(b)(6)

From: Denison, Mark (NIH)
Sent: Fri, 6 Jun 2014 20:13:17 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Baric, Ralph; (b)(6)
Subject: Greetings and Question about AI108197
Attachments: R01AI108197_Modified_Summary_Aims.pdf, R01AI108197_Response_to_Reviews.pdf

Dear Erik

We haven't had a chance to chat much this past year, but we (my lab and Ralph Barics) have made great progress on our joint PI grant AI 108197 (Determinants of Coronavirus Fidelity in Replication and Pathogenesis). Its time for the RPRR and I wanted to be sure to touch base with you.

I am just returning home from the Coronavirus (nidovirus) meeting in Spain, so still in transit in a hotel in Madrid. Thus cant talk in person until Monday.

I wanted to review something that was completed prior to your appointment as program officer for this grant, but which I don't see reflected on the grant page. It is based on the appended documents and the email (See below) we submitted last May 14 prior to the awarding of the grant in August. It is modified Aims and scope of work based on the reduced annual award and reduction from 5 to 4 years total.

I am basing our RPRR and ongoing plans on these modified AIMs and scope of work, which as you can see from the progress report and papers, is going very well with high impact publications and progress. I just wanted to be sure these get either updated on the commons site. If we need to have any conversation I am happy to do so.
I will be available Monday June 9 and would be happy to talk with you about it.

Thanks again, and I hope to get the chance to meet you in person soon

Best Regards

Mark

Mark R. Denison M.D.
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Professor of Pathology, Microbiology & Immunology
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Nashville, TN 37232-2581
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(cell)
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From: <Denison>, Mark Denison (b)(6)
Date: Tuesday, May 14, 2013 5:05 PM
To: "Salomon, Rachelle (NIH/NIAID) [E]" (b)(6)
Cc: David Spiro (b)(6) Ralph Baric (b)(6) "Grossman, Sonnie Kim (NIH/NIAID) [E]" (b)(6)
Subject: R01-AI-108197 Modified Aims and Response to Reviews

Dear Rachelle,

I am pleased to provide our modified Aims and Detailed Response to Reviews. I worked closely with Dr. Baric review the proposed budget modification and scope of work. We have modified the proposal to focus on the most high impact, timely and feasible studies achievable with the modified budget. In addition, our modifications are responsive to the comments in the reviews from VIRB study section. We believe that this will allow us to rapidly provide important new basic and translational discoveries applicable to both SARS-CoV and the emerging MERS-CoV.

Rachelle, I am copying to David and Sonnie as I am not sure whether you are back in the office.
Thanks to all of you for your good communication and help.

Best Regards

Mark

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Introduction to Modified Summary and Specific Aims for 1-R01-AI108197-01: The proposed modified budget will remove approximately $820,000 (23%) during a 5 year funding period. To maintain the impact, quality, and timeliness of the research in light of this reduction, we have modified Specific Aims as presented below. In addition, the modifications address the comments of the reviewers as described in the "Response to Reviews". We thank the NIH for recognizing the critical nature of this work and for supporting these highly innovative studies focusing on SARS-CoV and on the persistent, pathogenic emerging MERS-CoV. We look forward to rapid progress on this important virus of high public health concern.

Modified Summary. Emerging zoonotic coronaviruses (CoVs) have pandemic potential and cause significant mortality and social disruption, including SARS-CoV, and the current MERS-CoV that has resulted in greater than 50% mortality in reported cases. RNA virus zoonotic emergence and disease, as well as resistance to vaccines and antivirals, has long been held to result from generation of a "mutant swarm" or quasispecies of related mutants around the consensus genome, favoring rapid selection of adaptive variants. Until recently this was proposed to result solely from "error-prone" RNA-dependent RNA polymerases with low fidelity (high mutation rates) and lacking proofreading during RNA synthesis. CoVs contain the largest positive-strand RNA genomes, up to 32 kb, posing unique challenges to models of intrinsic low-fidelity replication. CoVs encode a DE-D-Dh family 3'-to-5' exoribonuclease in nonstructural protein 14 (nsp14-ExoN). Genetic inactivation of nsp14-ExoN results in viable mutants of CoV-MHV and SARS-CoV with high-level mutator phenotypes (20-fold increased mutation rate) *in vitro* and *in vivo*, as well as decreased fitness and stable attenuation in vivo. The data support the conclusion that nsp14-ExoN is the first discovered RNA-dependent RNA proofreading exonuclease, and is essential for CoV fidelity, virulence and pathogenesis. However, it is unknown how nsp14 regulates CoV replication fidelity. The specific aims of the proposal will; 1) define the determinants of altered fidelity in nsp14 of SARS-CoV and MERS-CoV and test the impact of altered fidelity on stability, fitness and resistance to RNA mutagens; 2) determine the effect of fidelity mutants in vitro on exonuclease and methyltransferase activity; and 3) establish the impact of altered fidelity in vivo in animal models on pathogenesis, stable attenuation and immune response. The results of the proposed experiments will identify conserved fidelity determinants in nsp14 critical for replication, pathogenesis and virulence of SARS-CoV and MERS-CoV, and identify broadly applicable approaches for zoonotic and other human CoV attenuation and targets for inhibition.

MODIFIED SPECIFIC AIMS. RNA virus host-species movement, adaptation, evolution, disease, and resistance to vaccines and antivirals is thought to result from a "cloud" of related mutants around a consensus genome, also referred to as quasispecies, resulting from low fidelity (high mutation rate) RNA-dependent RNA polymerases (RdRps) that lack proofreading during RNA synthesis. Coronaviruses (CoVs) are broadly distributed in humans, bats, and other mammals, and are adept at host-species movement and adaptation, as demonstrated by SARS-CoV and by the highly pathogenesis novel human CoV, **(HCoV-EMC, MERS-CoV)** that is causing >50% mortality. CoVs contain the largest RNA genomes, up to 32 kb in length, raising fundamental questions about how CoVs generate necessary population diversity while maintaining genomic stability. The Denison and Baric laboratories have shown that CoVs encode a DEDDh family 3'-to-5' exoribonuclease in nonstructural protein 14 (nsp14-ExoN), and that genetic Inactivation of ExoN (ExoN-) of murine hepatitis virus (MHV) and SARS-CoV results in a mutator phenotype with 20-fold decreased fidelity that is genotypically and phenotypically stable *in vitro* and *in vivo*. CoV ExoN- mutator viruses have impaired competitive fitness compared to wildtype (ExoN+) viruses, are attenuated in lethal SARS-CoV mouse models, and do not revert to virulence. Thus all data indicate that CoVs encode the first known RNA-dependent RNA proofreading enzyme that is a critical regulator of fidelity and diversity. However, It is not known how nsp14-ExoN regulates fidelity. Experiments in this proposal will test the overall hypothesis *that nsp14 contains multiple determinants that increase or decrease replication fidelity, and that genetic alteration of fidelity that increases or decreases mutation rate and population diversity impairs fitness, and the virus capacity to mediate pathogenesis.* The specific aims of the proposal will identify determinants conserved across different CoV nsp14-ExoN that regulate fidelity and define the impact on replication, exonuclease activity, virulence, and pathogenesis. The specific aims of the proposal build on the complementary strengths and collaborations of the Denison and Baric labs in MHV and SARS-CoV reverse genetics, replicase protein mutagenesis and functions, pathogenesis, immune response, and synthetic genomics. The results of the proposed experiments will identify fidelity determinants, establish the range of tolerated increased and decreased fidelity, and identify novel approaches and targets for attenuation and inhibition of **SARS-CoV and MERS-CoV**.

Aim 1. To define nsp14 fidelity determinants and their impact on SARS-CoV and MERS-CoV replication and fitness. In **part 1,** we will use MHV and SARS-CoV to test the effect of predicted and systematic mutations in nsp14-ExoN motifs and residues, Zn finger domain, conditional (ts) alleles, conserved charged residues outside of the ExoN motif, and the carboxy-terminal N7-methyltransferase domain in nsp14 on replication fidelity by next generation sequencing and mutagen sensitivity. Experiments in **part 2** will test the impact of altered fidelity on virus genotypic and phenotypic stability and competitive fitness during infection and passage in culture. In **part 3** we will use the newly established reverse genetic system for **MERS-CoV** to test for conservation of ExoN mediated fidelity and fidelity altering mutations on replication in multiple continuous and primary cell lines of the human lung.

Aim 2. To define the effect of nsp14-ExoN fidelity altering mutations on RNA synthesis, and on exonuclease and N7-methyltransferase activity in vitro. In **part 1** we will determine the effect of increased and decreased fidelity mutations on RNA synthesis and recombination **for SARS-CoV and MERS-**CoV. In **part 2,** we will determine the in vitro biochemical mechanism of activity of altered fidelity mutations in vitro on nsp14 Exonuclease and N7-methyltransferase activity. In **part 3** we will determine the sensitivity of nsp14 mutants to RNA mutagens, nucleoside analogs and β-IFN, testing the mechanism action during infection.

Aim 3. To determine the effect of altered fidelity on *in vivo* replication and pathogenesis. We will test the *hypothesis that decreased or increased fidelity is attenuating for SARS-CoV and MERS-CoV replication and pathogenesis in vivo, while allowing protective immune response*. In **part 1** we will use selected increased and decreased fidelity mutants to test replication and pathogenesis in aged, immunocompromised and persistently infected mice of different genetic backgrounds. In **part 2,** we will determine minimal lethal dose, lung pathology, tissue tropism and effects on respiratory function in young and aged mice, in order to define the limits of fidelity regulation on in vivo pathogenesis in the lung. **In part 3,** we will test increased and decreased fidelity mutants during in vivo passage for genotypic and phenotypic stability and reversion to virulence. In **part 4** we will apply results from **parts 1-3** in animal models of **MERS-CoV** to test conserved attenuating ExoN fidelity mutants on replication, pathogenesis, immune response and stability.

ORIGINAL SPECIFIC AIMS

SPECIFIC AIMS. Emerging human and zoonotic RNA viruses like SARS-CoV cause significant global morbidity, mortality, and social disruption. The current paradigm for RNA virus host-species movement, adaptation, evolution, disease, and resistance to vaccines and antivirals is based on the generation of a vast diversity of related mutants around a consensus genome, also referred to as quasispecies or "mutant swarms", that allow for rapid adaptation under selective pressure. In this model, viral population diversity results from low fidelity (high basal mutation rate) RNA-dependent RNA polymerases (RdRps) that lack of error recognition and repair - or proofreading - during RNA synthesis. Coronaviruses (CoVs) are broadly distributed in humans, bats, and other mammals and avian species, and are adept at host-species movement and adaptation, as demonstrated by SARS-CoV and by the recent identification in the Middle East of a novel human CoV, HCoV-EMC/2012, since both are likely zoonoses from bats. However, CoVs contain the largest and most complex positive-strand RNA genomes known, up to 32 kb in length, posing unique challenges to models of intrinsic low-fidelity replication, and raising fundamental questions about how CoVs generate necessary population diversity while maintaining genomic stability. The Denison and Baric laboratories have collaborated to demonstrate that CoVs encode a DEDDh family 3'-to-5' exoribonuclease in nonstructural protein 14 (nsp14-ExoN) that is required for CoV replication fidelity. Mutations that inactivate ExoN (ExoN-) of murine hepatitis virus (MHV) and SARS-CoV result in a mutator phenotype with 20-fold decreased fidelity that is genotypically and phenotypically stable *in vitro* and *in vivo*. CoV ExoN- mutator viruses manifest a loss of competitive fitness compared to wildtype (ExoN+) viruses, are profoundly sensitive to inhibition by RNA mutagens, are attenuated in lethal SARS-CoV mouse models, and do not revert to virulence. By encoding a fidelity-enhancing DEDDh ExoN, CoVs challenge the paradigm that RNA viruses do not proofread. It is not known how nsp14-ExoN regulates fidelity. ExoN activity is in a protein distinct from the RNA dependent RNA polymerase (nsp12) and also has been shown to be stimulated in exonuclease activity *in vitro* by CoV nsp10. The contributions of these and other CoV proteins to CoV fidelity regulation also is unknown. Experiments in this proposal will test the overall hypothesis *that CoVs encode multiple proteins, including nsp14, nsp12, and nsp10, that together constitute a fidelity complex that regulates replication fidelity*.

The specific aims of the proposal will identify determinants conserved across different CoV nsp14-ExoN that regulate fidelity, test for the contributions of other replicase proteins in fidelity, and to define the impact on replication and pathogenesis. The specific aims of the proposal build on the complementary strengths and collaborations of the Denison and Baric labs in MHV and SARS-CoV reverse genetics, replicase protein mutagenesis and functions, pathogenesis, immune response, and synthetic genomics. The results of the proposed experiments will identify fidelity determinants, establish the range of tolerated fidelity, and identify novel approaches and targets for attenuation and inhibition.

Aim 1. To define nsp14 fidelity determinants and their impact on fitness and RNA synthesis. We will test the *hypothesis that conserved determinants within nsp14 can increase or decrease replication fidelity.* In **part 1,** we will test the role of nsp14-ExoN motifs and residues, the novel Zn finger domain, conditional (ts) alleles, conserved charged residues outside of the ExoN motif, and the carboxy-terminal N7-methyltransferase domain in nsp14 on replication fidelity. Experiments in **part 2** will test the impact of altered fidelity on competitive fitness. In **part 3,** we will determine effect of mutations that alter fidelity on viral RNA synthesis.

Aim 2. To identify proteins and determinants in the CoV fidelity complex. We *hypothesize that nsp14-ExoN proofreading functions within a fidelity complex that includes nsp12-RdRp and nsp10.* In **part 1**, we will generate and test viruses with mutations in nsp10 that are: known to stimulate nsp14-ExoN activity *in vitro*; affect viral replication or RNA synthesis; are temperature sensitive; or are conserved charged residues. Studies in **part 2** will use a modeled structure of nsp12-RdRp to design and test mutations predicted to alter fidelity. Nsp12-altered fidelity mutants will be tested in combination with mutants in nsp14 and nsp10. In **part 3**, we will use the RNA mutagen 5-fluorouracil (5-FU) and the non-obligate chain terminator 2'-C-methyladenine to select for resistance in ExoN- and ExoN+ viruses and identify and test candidate resistance mutations for impact on fidelity in ExoN+ and ExoN- virus backgrounds.

Aim 3. To determine the effect of altered fidelity on *in vivo* replication and pathogenesis. We will test the *hypothesis that decreased or increased fidelity is attenuating for CoV replication and pathogenesis in vivo, while preventing reversion to virulence and allowing protective immune response*. We will use a select subset of increased and decreased fidelity mutants in the mouse-adapted SARS-CoV (SARS-MA) background to test: **part 1**, replication and pathogenesis in aged and immunocompromised mice of different genetic backgrounds; **part 2**, susceptibility to reversion to virulence; and **part 3**, immunogenicity and protection from lethal challenge.

1-R01-AI108197-01 Principal Investigators. Denison, M.; Baric, R.

Response to Summary Statement Review. We are pleased to respond to the comments from the Study section with new data, clarifications and responses. In response to budget modifications, the scope of work has been modified to focus on nsp14 of SARS-CoV and MERS-CoV, as described in modified Summary and Specific Aims. The responses reflect this focus to achieve the goals of the proposal and the important new research on MERS-CoV. The responses are inclusive of all comments.

Comment: *The proposed work will "not get at mechanisms".*
Response: We apologize for not clearly presenting the scope of the research and note that the program is designed to specifically determine the genetic determinants and proteins that regulate fidelity. These studies will identify the contribution determinants in nsp14 on fidelity function for both SARS-CoV and MERS-CoV (Aim 1) and now the mechanism by which altered fidelity affects RNA synthesis and recombination (Aim 2). In addition, Aim 2 now will test in vitro exonuclease and N7-methyltransferase activity of nsp14 mutants. This will address mechanism specifically.

Comment: *An intrinsic weakness is that the structure of the replication complex is not known, which will make it difficult to interpret some of the data obtained in Aim 2. Interpretation of introduced mutations may be difficult in the absence of this information.*
Response: Our studies have in fact significantly galvanized the interest of several excellent structural biologists. Because of our findings on fidelity regulation, several groups are working on the structure of nsp14 alone and in complex with other viral replicase proteins. Specifically, we have added approaches in Aim 2 to define in vitro expression and testing of nsp14 mutants for ExoN and N7-MT activity. We will use these studies as the basis for collaboration with Dr. Peersen on high-probability models for nsp14-ExoN and N7-MT. Although within the direct scope of the proposed work, we will work to identify collaborators interested in structure determination. We will integrate any newly discovered structure-based insights into our biological, molecular and biochemical models and use this information to inform second-generation mutant design.

Comment: *In absence of structural information about the putative replication complex, it may be difficult in to distinguish requirements for nsp14 function from changes in conformation that indirectly impact fidelity.*
Response: We respectfully submit that this is an artificial distinction, since ***both*** conformation and specific alleles are essential for nsp14 function in fidelity regulation. We propose that our studies in modified Aim 2 will identify the role of activity (e.g. nucleotide recognition and excision) and protein dynamics and interactions. This diversity of possibilities will define broadly how CoVs assemble and use fidelity regulation to achieve goals of propagation and pathogenesis. In modified Aim 2, we will distinguish structural disruption of nsp14 from specific effects on fidelity by monitoring the activity of the N7-MTase encoded in the carboxy-terminal half of nsp14. Recent studies have demonstrated that ExoN and N7-MTase activity are functionally linked, thus conformational perturbations of ExoN would likely prevent interaction with N7-MTase and affects its function, while functional mutations in the proofreading activity, should not alter the binding interface with N7-MTase, nor alter its function. Reduced N7-MTase activity would increase virus sensitivity to β-IFN, since the N7-MTase activity is required for 2'-O-MTase.

Comment: *Further rationale for Aim 3 should be provided. Studies analyzing reversion may reveal novel mutations not observed after passage in culture. The remainder of this aim appears to be incremental.*
Response: It was not previously known how decreasing or increasing CoV fidelity affects pathogenesis in vivo, because *this is the first viral system capable of testing that question.* Nor is it known what the tolerated magnitude of fidelity dysregulation is for replication or pathogenesis. The goal of modified Aim 3 is to use both increased and decreased fidelity mutants to determine the range of fidelity changes tolerated in vivo; and to identify the optimal fidelity dysregulation that balances replication in vivo with attenuation and protection. In vivo studies of Aim 3 are critical, as this is the context that fidelity acts in. To date we have tested only the highly impaired ExoN-, and the studies were focused on virulence and protection, not detailed pathogenesis. In addition, in Aim 3, we will test conserved fidelity mutants defined in MERS-CoV background as we or others define animal models, in order to define the broad applicability of approaches to a currently emerging, highly-pathogenic human CoV. This profoundly increases the impact, timeliness and translational potential of the results.

In response to the comment that the aim is “incremental”, we could not disagree more emphatically! This statement is at odds with NIH positions on basic and translational research and bio-preparedness for emerging infections and zoonotic pathogens. The only “existing data” in this field comes from our labs and that of our collaborator Marco Vignuzzi on the impact of decreased fidelity on replication and virulence. The finding first that proofreading occurs, and second that decreased fidelity is stably attenuating, are profound new ideas, with direct applicability to a current emerging zoonotic pathogen with significant pandemic potential.

Comment: *This protein (nsp14) is found only in coronaviruses and may be limited to a special replication complex.*
Response: We agree – and would argue that this is the key important point of the proposal. For global health response to the current and highly probable future zoonotic CoVs, the presence of a novel proofreading enzyme in represents a unique, highly-specialized, non-redundant, virus-specific and vulnerable target for therapeutic intervention and vaccine design that can be applied to the current SARS and MERS-CoV viruses as well as future emerging outbreaks or applied to “common” strains that suddenly evolve increased pathogenic potential. We would argue that there is profound significance to a unique vulnerability in a virus or virus family. Supporting the importance of “limited” proteins, we cite the example of reverse transcriptase of retroviruses, that has provided a robust target for therapeutic design. Finally, while ExoN may be unique to the large nidoviruses (including CoVs), *emerging data from the Vignuzzi lab and others support the conclusion that 2-4 fold RNA-dependent RNA polymerase fidelity regulation is a universal feature of RNA viruses; these studies are so recent that it is premature to argue that other RNA viral proteins/cistrons do not contribute to fidelity regulation as well*. Our studies demonstrate this possibility. Thus, insights from our studies will inform studies of the relationship of fidelity to pathogenesis in other RNA virus families.

Comment: *The studies are based on in part on previously generated mutants that demonstrated that such mutants could replicate in the animal model.*
Response: This comment was in the “weakness” category, but we consider this a strength and demonstration of feasibility. Our previous studies provided the first evidence that altered fidelity reduces CoV fitness in vivo. They also provided the critical framework for using altered fidelity viruses as CoV vaccines. The ability of the ExoN- virus to replicate in animal models, both acute and persistent, provides a uniquely powerful model for testing a range of fidelity mutants and exploring new ways to extinguish virus replication in Aim 3. As an example, understanding the limits of viable fidelity alteration will allow targeting of potential inhibitors to recapitulate the genetic phenotype by driving the virus to extinction.

Comment: *Aim 3 is somewhat dependent on useful mutations from Aims 1 and 2 and maybe limited to the deletions already generated if no useful mutants come from these aims.*
Response: We are confident that we will recover viable mutants from nsp14, since we already have mutants in other nsp14 ExoN motifs, *ts* alleles, and N7-MT domain. In addition, with the availability of the MERS-CoV clone, we will rescue this conserved mutations in that background. That will represent a significant target for studies in Aim 3 while we rescue other mutations in nsp14. To demonstrate feasibility, we note that the coronavirus reverse genetic systems for MHV, SARS-CoV and MERS-CoV used in this proposal were developed by the Baric and Denison laboratories, and we have successfully generated hundreds of mutants over the past 10 years that have been used to probe CoV replication, pathogenesis, evolution, vaccine design, and therapeutics, as well as probing the limits of viral host-species movement. Importantly, we recovered the ExoN- mutant as a highly replication competent mutant, *in spite of results from other groups concluding that it was a lethal mutation preventing virus recovery in CoVs.*

Comment: *While the genetic experiments will be informative, consideration of additional innovative biochemical experiments will further help define the nature of the putative proofreading complex and interpret mutant phenotypes, esp. given the absence of high-resolution structures of the relevant coronavirus proteins.*
Response: The reviewer is correct that biochemical assays that measure the impact of targeted mutations on fidelity functions or on the specific enzymatic activities of nsp14 are of high value. We have focused our overall studies on nsp14 and have expanded studies in modified Aim 2 to test the effect of fidelity altering mutations and other mutations in nsp14 on in vitro exonuclease activity and N7-methyltransferase activity.

Comment: *The so-called “resistance to reversion to virulence” property of the fidelity mutants needs to be*

interpreted with caution. It is conceivable that some low fidelity mutants could gain increased fidelity (e.g., by repairing ExoN itself or other interacting factors) and virulence, especially in a setting of large population infection as would occur with vaccination.

Response. We appreciate this comment and apologize for not presenting this concept more clearly. A major goal of the entire program is to identify the effective range of altered fidelity during coronavirus infection in vitro and in vivo. We agree that mutations with low altered fidelity defects may revert or be fully pathogenic, so proof of this paradigm-setting hypothesis is dependent on evaluating a suite of mutants with low and high fidelity. Thus, Aim 3 is both highly significant but critical to the entire goals of the program, since translational impact of this approach is dependent on our ability to carefully define the parameters of fidelity alteration and its impact on in vivo pathogenesis. The concept of altered fidelity (increased or decreased) as an attenuating mechanism is very new; however it is being demonstrated in other viruses as well (alphaviruses, picornaviruses). *It is our hypothesis rather than a conclusion* that altered fidelity is is stably attenuating. Our result with ExoN- attenuation, while unprecedented, supports the conclusion that fidelity is a unit of evolutionary selection that is essential for all stages in the virus life cycle in natural hosts. We will apply very rigorous standards to newly identified mutants. Those that show reversion to virulence will be sequenced and tested for fidelity and mechanism of reversion. Any identified revertant viruses with specific mutations will be highly informative for understanding both intramolecular (nsp14) and likely intermolecular interactions and determinants of fidelity.

From: Aleksei Chmura
Sent: Thu, 10 Aug 2017 10:58:41 -0400
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Dr. Peter Daszak; Alison Andre; Lu, Kristina (NIH/NIAID) [E]; 李泓萤
Subject: Re: Invitation to US-Japan 20th Int'l Conference on Emerging Infectious Diseases - NIAID
Importance: High

Dear Erik,

Apologies for this. It looks like Kristina's email may have been missed. Peter is out of the office until next week, but we will respond now to Kristina's email.

Cheers!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Aug 9, 2017, at 15:11, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Peter and Aleksei,
A colleague of mine, Dr Kristina Lu, has reached out regarding a US-Japan emerging infectious diseases conference she is organizing. She was hoping to invite Peter to speak on emerging CoVs in Asia. We would appreciate it if you could please let us know, even if you're not available to attend.

Many thanks,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18

Bethesda, MD 20892-9825
Phone: (b)(6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Lu, Kristina (NIH/NIAID) [E]
Sent: Wednesday, July 12, 2017 8:37 AM
To: (b)(6)
Cc:
Subject: Re: Invitation to US-Japan 20th Int'l Conference on Emerging Infectious Diseases - NIAID

Dear Dr. Daszak,

I'm following-up on my invitation below.

Thanks again!

--Kristina

From: "Lu, Kristina (NIH/NIAID) [E]" (b)(6)
Date: Thursday, June 29, 2017 at 6:20 PM
To: (b)(6)
Subject: RE: Invitation to US-Japan 20th Int'l Conference on Emerging Infectious Diseases - NIAID

Dear Dr. Daszak,

Just following-up on my invitation.
Unfortunately, I wasn't able to attend your DMID Forum presentation, so I couldn't touch base with you in-person.

Many thanks!

--Kristina

From: Lu, Kristina (NIH/NIAID) [E]
Sent: Thursday, June 22, 2017 7:31 PM
To: (b)(6)
Subject: Invitation to US-Japan 20th Int'l Conference on Emerging Infectious Diseases - NIAID

Dear Dr. Daszak,

I would like to invite you to the **U.S.-Japan Cooperative Medical Sciences Program (USJCMSP) 20th International Conference on Emerging Infectious Diseases (EID)** and the **20th Acute Respiratory Infections (ARI) Panel Meeting** in **Shenzhen, China** during **January 8-12, 2018**. I am the Secretariat for the US-Japan ARI Diseases Panel and a Program Officer at NIAID-NIH. I work with Erik Stemmy (NIAID), who highly recommended you for participation and presentation.

The focus of this conference will be on pathogenesis and immunity of viral diseases of importance in the Asia-Pacific region. The conference objectives are to share current research findings and foster existing and potential international research collaborations that engage investigators and institutions in the Asia-Pacific region and the United States.

https://www.niaid.nih.gov/research/us-japan-cooperative-medical-science-program-organization-and-history

During the EID Conference, there will be broad coverage on a number of viral diseases, including influenza, ebola, HIV, dengue, zika, and hepatitis. In conjunction with the EID Conference, the ARI Panel Meeting will convene with a more focused agenda on emerging virus diseases at the animal-human interface, including influenza and coronaviruses.

I am hoping you are willing to give two presentations on the following topics –

1. EID - pathogenesis / trends of CoVs in Asia
2. ARI - there is flexibility for a presentation topic of your choice

We will support your travel expenses.

Please let me know if you are able to participate and present. Many thanks in advance and looking forward to hearing from you!

Kind regards,
Kristina

****
Kristina T. Lu, PhD
Program Officer
Respiratory Diseases Branch
Division of Microbiology & Infectious Diseases
NIAID | NIH | DHHS

Phone: (b)(6)
(b)(6)

From: Peter Daszak
Sent: Wed, 2 Aug 2017 16:55:15 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Automatic reply: Potential visit to NIH by our Chinese Co-investigator in June?

I'll be out of the office until August 15th, and then from August 17th-22nd and will not have access to email.

Please cc Alison (b)(6) on all emails and I'll respond as soon as possible when I'm back.

Cheers,

Peter

From: Chen, Ping (NIH/NIAID) [E]
Sent: Fri, 28 Jul 2017 22:25:10 +0000
To: Peter Daszak
Cc: Stemmy, Erik (NIH/NIAID) [E]; Hongying Li; Aleksei Chmura; Alison Andre
Subject: Re: Meeting in Beijing on September 4-5?

Hi Peter,

Sept 4 is the Labor Day. I should be in the office on the 5th.

Looking forward to seeing you.

Ping

Sent from my iPhone

On Jul 29, 2017, at 5:37 AM, Peter Daszak (b)(6) wrote:

Hi Ping,

Hope everything is well.

Dennis Carroll from USAID and I will be in Beijing on September the 4th and 5th for the Global Virome Project initiate in China.

There has been progress since the committee meeting in Beijing this February, and we wanted to meet with the Chinese Academy of Sciences and other stakeholders at the US Embassy for more discussions. Would you be in Beijing and available to join some of our meetings.

I've cc'd my NIAID program officer, Erik Stemmy, as well to keep him in the loop.

I look forward to hopefully seeing you in Beijing.

Cheers,

Peter

Peter Daszak
President

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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Aleksei Chmura
Sent: Mon, 10 Jul 2017 15:37:00 -0400
To: Kurilla, Michael (NIH/NIAID) [E]
Cc: Dr. Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]; Cassetti, Cristina (NIH/NIAID) [E]; Addison, Raynita (NIH/NIAID) [E]
Subject: Re: Potential CEPI overlap projects - can we discuss?

Dear Michael,

Peter is just calling-in. Apologies for the delay, but cell service in DRC is spotty and he is just getting to a landline.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (direct)
(mobile)
(b)(6) (Skype)

www.ecohealthalliance.org

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On Jul 10, 2017, at 15:16, Kurilla, Michael (NIH/NIAID) [E] (b)(6) wrote:

Raynita had to leave early today.
Let's use: (b)(6)
Passcode:

Michael G Kurilla, MD-PhD
Director, Office of BioDefense, Research Resources, and Translational Research
Associate Director for BioDefense Product Development
DMID, NIAID, NIH, DHHS
5601 Fishers Lane 8G61
Rockville, MD 20852
(b)(6)

Death: "Humans beings make life so interesting. Do you know, that in a universe so full of wonders, they have managed to invent boredom."

- *Terry Pratchett, from Hogfather*

From: Aleksei Chmura (b)(6)
Sent: Saturday, July 08, 2017 6:05 PM

To: Kurilla, Michael (NIH/NIAID) [E] (b)(6)
Cc: Dr. Peter Daszak (b)(6) Addison, Raynita (NIH/NIAID) [E] (b)(6)
Subject: Re: Potential CEPI overlap projects - can we discuss?
That will be super! I will set up the call with Raynita.
Cheers,
-Aleksei

On Jul 8, 2017, at 17:59, Kurilla, Michael (NIH/NIAID) [E] (b)(6) wrote:

Monday after 3:30PM my time works.
Raynita can arrange for me.
Michael G Kurilla, MD-PhD
Director, Office of BioDefense, Research Resources, and Translational Research
Associate Director for BioDefense Product Development
DMID, NIAID, NIH, DHHS
5601 Fishers Lane 8G61
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Death: "Humans beings make life so interesting. Do you know, that in a universe so full of wonders, they have managed to invent boredom."
- *Terry Pratchett, from Hogfather*

From: Aleksei Chmura (b)(6)
Sent: Friday, July 07, 2017 10:14 PM
To: Kurilla, Michael (NIH/NIAID) [E] (b)(6)
Cc: Dr. Peter Daszak (b)(6)
Subject: Re: Potential CEPI overlap projects - can we discuss?
Importance: High
Dear Michael,
Would any blocks of time from the following US (East Coast) times work for you?
Monday 10th July: 12:00pm - 5:00pm
Tuesday 11th July: 3:00pm - 4:00pm
Once you confirm, I will send around call-in details.
Sincerely,
Aleksei Chmura
Senior Coordinator of Operations

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From: Peter Daszak
Sent: Friday, July 7, 2017 8:19 PM
To: Kurilla, Michael (NIH/NIAID) [E]
Subject: RE: Potential CEPI overlap projects - can we discuss?

Definitely. I'm traveling but am only 4 hours ahead and can set up a time. I've cc'd Aleksei who will be able to coordinate based on my flights etc. and when you're available. Monday or Tuesday would be good…

I've also been thinking about CEPI, given our recent findings of SARS-like viruses from bats that are so close to SARS-CoV that they cause similar clinical signs in the humanized mouse model, but when you treat with a monoclonal that knocks SARS-CoV, it has zero effect on the bat virus….It implies that CEPI could lay out funds for vaccine development that might not be effective against the broader array of undiscovered viruses out there.

Anyway – look forward to talking with you..

Cheers,

Peter

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From: Kurilla, Michael (NIH/NIAID) [E] (b)(6)
Sent: Friday, July 7, 2017 6:28 PM
To: Peter Daszak
Subject: Potential CEPI overlap projects - can we discuss?

Peter,

For a couple of reasons, I'm looking to develop some small scale projects related to CEPI that might have some overlap with ongoing or planned GVP activities.

For example, we are discussing some surveillance and screening activities in eastern Africa for MERS-like coronaviruses and Nipah viruses in southeast Asia. The idea would be to collect various environmental samples as well as human blood specimens to look for viruses and serologic evidence of exposure, respectively with a longer term goal of perhaps isolating human MAbs.

Would you have time to discuss early next week?

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5601 Fishers Lane 8G61
Rockville, MD 20852
(b)(6)

Peace is not found in a calmer storm; it's found in a better boat.

- *Travis Meadows*

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thu, 29 Jun 2017 18:48:22 +0000
To: Peter Daszak
Cc: Hongying Li
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Thanks Peter. It was great seeing you and meeting the team. I'll forward this to our communications office.

Erik

From: Peter Daszak (b)(6)
Sent: Thursday, June 29, 2017 2:39 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Erik,

I just wanted to say thanks for hosting us at NIAD today – it was great to have an interested audience with good questions and nice to have a chance to introduce our collaborators to you personally.

I mentioned the upcoming SADS-CoV paper might get into *Nature*. Obviously, this is touch-and-go right now, but I've attached the draft here so you can forward it to your communications team in case they want to get a release out earlier this time.

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Hope you enjoy skimming through them, and thanks again for setting up the talk this morning.

Cheers,

Peter

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Sent: Thursday, June 29, 2017 7:22 AM
To: Peter Daszak
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Also, please let me know when you arrive at security and I'll meet you there. My mobile is (b)(6) (b)(6)

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From: Peter Daszak (b)(6)
Sent: Thursday, June 29, 2017 12:43 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Hongying Li (b)(6)
Cc: Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Erik,

In case NIAID has issues with USB drives etc., here is a pdf version of our talk for tomorrow morning. I hope you can have that as a backup from your email in case we can't download our talk from our laptops.

Look forward to seeing you.

Cheers,

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, June 26, 2017 9:30 AM
To: Hongying Li
Cc: Peter Daszak; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Thank you Hongying. I will forward it to security. Looking forward to your visit later this week.

Erik

From: Hongying Li (b)(6)
Sent: Monday, June 26, 2017 9:25 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Peter Daszak (b)(6) Aleksei Chmura (b)(6)
Alison Andre (b)(6)
Subject: Re: Potential visit to NIH by our Chinese Co-investigator in June?

Dear Erik,

Not sure if this is too late, but wanted to send you the updated attendee information with Peng Zhou's visa number. Please find it in the attachment. Let me know if there is any question.

Thanks,
Hongying

On Jun 16, 2017, at 11:22 AM, Hongying Li (b)(6) wrote:

Dear Erik,

Please find the security screening information for Zhengli Shi, Peng Zhou, and Hongying Li in the attachment. We don't have the visa No. for Peng Zhou at this moment because his visa application is still under administrative processing at the Embassy. We are not sure if he can obtain his visa on time or not, but will let you know as soon as we have any further confirmed information.

Please let me know if there is any question. Thank you!

Best,
Hongying

<5601 Foreign Visitor Form-China.xlsx>
On May 24, 2017, at 3:16 PM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Peter,
Thanks for this information. I've attached a form that will help expedite security screening for Dr Zhou and Hongying Li. Can you please have them complete the information on the second sheet of the attachment? I'll need to turn it in to our security office at least a week before your visit, so if you could get it back to me by June 19th or 20th that would be great. Also, please let them know they should bring their passports with them. Everyone else will need a photo ID as well.

Let me know if you need directions to our building. I would suggest planning to arrive between 8:15 and 8:30, as there can be a line at security if there are other public meetings occurring that day. There is no visitor parking at our facilities, but there is a public parking garage on our block that I can get validation stickers for if you'll be driving. We are also a short walk from the Twinbrook Metro stop, if you plan to travel by train.

Erik

From: Peter Daszak (b)(6)
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To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?
Importance: High

Hi Erik,

Great to hear from you and looking forward to the talk on June 29th

We're proposing for 4 people to visit NIAID and I've attached bios for all of them to this email. Note that Dr Shi, Dr. Zhou and Hongying Li are all Chinese nationals, and I'm not sure what sort of clearance you'll need for that, so please let me know and we'll work on getting the relevant documents to you

1. Myself, PI on the NIAID CoV grant, President of EcoHealth Alliance, EHA lead on the USAID PREDICT project
2. Dr. Zhengli Shi, Co-Investigator on the NIAID CoV grant, Director of Center for Emerging Diseases at The Wuhan Institute of Virology
3. Dr. Peng Zhou, Associate Professor at Wuhan Institute of Virology
4. Hongying Li, Research Scientist and Country Liaison for China at EcoHealth Alliance

Re a title for the talk, bearing in mind it should be broader than just SARS-CoV, what about the following:

"SARS, MERS and the risk of novel viral emergence from bats"

Zhengli and I will do a double act, and we'll cover the work we're doing on the NIAID project, as well as the broadscale surveillance of bats for novel viruses in PREDICT.

Cheers,

Peter

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To: Peter Daszak
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Peter,

We've got you on the calendar for June 29th. Can you send me a title for the talk, short summary, and brief bios for the presenters?

Thank you!
Erik

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Sent: Monday, April 24, 2017 4:47 PM

To: Peter Daszak (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Ok! I'll see about scheduling you for the slot on June 29th. Can you send me a title and short synopsis? Since our whole division would be attending it would be great if you could cover some of the collaborative work with PREDICT and not solely focus on the MERS work.

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To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

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We'll plan to come to DC the afternoon or evening before and then do the symposium and meet with you.

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Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Peter,

I would be happy to have you visit us in June. I am available on the 28th. If there is any flexibility in your schedule, Thursday mornings we have a division-wide seminar from 9-10am, and that would be an ideal time to have you present on your work to the larger audience. I understand if that's not possible, thought, but thought I would check to see. Please let me know.

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Zhengli's timeline is fixed, and I wondered if you and your colleagues would be available on Wednesday June 28th? If not, we can look at alternative dates…

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<5601 Foreign Visitor Form.xlsx>

Hongying Li, MPH 李泓萤
China Programs Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (U.S. mobile)
(b)(6) (China mobile)
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From: Peter Daszak
Sent: Thu, 29 Jun 2017 18:38:50 +0000
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Hongying Li
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?
Attachments: 2017-05-06890 full manuscript.pdf

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(b)(6) (WeChat)

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Title: Fatal Swine Disease Outbreak Caused by a Novel Coronavirus of Bat Origin

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Spillover of bat-origin coronaviruses is implicated in the emergence of two emerging, high-impact zoonoses, SARS and MERS. Here, we report virological, epidemiological and experimental infection evidence that a novel bat-origin coronavirus, Swine Acute Diarrhea Syndrome coronavirus (SADS-CoV), caused an ongoing outbreak of lethal diarrheal disease in pigs in China. The outbreak began in January 2017 Guangdong Province in the vicinity of the origin of the SARS pandemic in 2002, and has caused the death of 24,693 piglets in four farms to date. We identified SADS related-CoVs with 96-98% sequence identity to SADS-CoV in 11.9% (71/596) of anal swabs collected from bats in Guangdong Province during 2013-16, predominantly in five *Rhinolophus* spp. horseshoe bats that are known reservoirs of SARS-like CoVs. The geographic, temporal, ecological and etiological similarities in the emergence of SADS and SARS highlight the urgent need to identify coronavirus diversity in bats to mitigate future outbreaks that threaten veterinary production, public health and economic growth.

The emergence of severe acute respiratory syndrome in southern China in 2002, which was caused by a previously unknown coronavirus (SARS-CoV)[1-5] and led to more than 8,000 human infections and 774 deaths [http://www.who.int/csr/sars/en/], heralded two new frontiers in emerging infectious diseases. Firstly, it demonstrated that coronaviruses are capable of causing fatal diseases in humans. Secondly, the identification of bats as the reservoir for SARS-related coronaviruses, and likely

origin of SARS-CoV[6-8] firmly established bats as an important source of highly lethal zoonotic viruses, which include Hendra, Nipah, Ebola and Marburg viruses[9].

The public health threat posed by novel coronaviruses was reinforced by the emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012[10], which has so far infected 1,952 people with 693 deaths [http://www.who.int/emergencies/mers-cov/en/]. Cases of MERS have been reported in 27 countries, mostly due to imported cases with the exception of a major outbreak in Seoul in 2015 that involved extensive local human-to-human transmissions[11]. While dromedary camels have been identified as the main source of MERS-CoV spillover to humans[12], there is evidence suggesting that bats are the original wildlife reservoir. This includes short sequence from a single *Taphozous perforatus* bat in Saudi Arabia, and evidence that bat MERS-related coronaviruses use the same human entry receptor, dipeptidyl peptidase 4 (DPP4; also known as CD26), as MERS-CoV[13-16].

Here we report a series of fatal swine disease outbreaks in Guangdong Province, China, approximately 100 km from the location of the purported index case of SARS. Most strikingly, we found that the causative agent for this swine acute diarrhea syndrome (SADS) is a novel coronavirus which is almost 99% identical in genome sequence to a bat coronavirus we detected in 2016 from a bat cave in the vicinity of the index pig farm. This new virus (SADS-CoV) thus appears to have originated from the same genus of horseshoe bats (*Rhinolophus*) as SARS-CoV.

From 28 October 2016, fatal swine disease outbreaks were observed in a pig farm in Qingyuan, Guangdong Province, China, very close to the location of the first known index case of SARS in Foshan (**Extended Data Figure 1**). Porcine epidemic diarrhea virus (PEDV) had caused prior outbreaks at this farm, and was detected in the intestine of deceased piglets at the start of the outbreak. However, PEDV could no longer be detected in deceased piglets after 12th January 2017, despite accelerating mortality (**Fig. 1**A) and extensive testing for other common swine viruses yielded negative results (**Extended Data Table 1**). These findings suggested an outbreak of a novel disease, which we designated swine acute diarrhea syndrome (SADS). Clinical signs are similar to those caused by other known swine enteric coronaviruses [17,18] and include severe and acute diarrhea, and rapid weight loss, leading to death due to nutritional exhaustion in newborn piglets less than four days of age. Infected piglets died 2-6 days following disease onset, while infected sows suffered only mild diarrhea and most recovered in two days. The disease caused no signs of febrile illness in piglets or sows. The disease has spread to three additional pig farms within 20-150 km of the index farm (**Extended Data Figure 1**) and, as of 2nd May 2017, has resulted in the death of 24,693 piglets from four farms (**Fig. 1A**). In Farm A alone, 64% (4659/7268) of all piglets born in February died.

Small intestinal samples from diseased piglets were taken from all four affected farms and subjected to next generation sequencing (NGS) using the Illumina MiSeq platform. Of the 338,036 total reads obtained, 369 mapped to viruses within the NCBI virus database, and 355 (96.2%) of these matched sequences of bat CoV

HKU2, a virus first detected in Chinese horseshoe bats in Hong Kong and Guangdong Province, China[19]. By *de novo* assembly and targeted PCR we sequenced a 27,173-bp coronavirus genome that shared 95% sequence identity to HKU2 (Genbank accession number NC009988.1). Four genomes of SADS-CoV were obtained, designated A, B, C and D corresponding to the four farms from which they were derived. These viruses are 99.9% identical to each other (**Extended Data Table 2**) (GenBank accession number: MF094681−MF094684), suggesting that inter-farm transmission was likely responsible for outbreaks on farms B, C and D.

Using quantitative PCR based on the nucleocapsid protein gene (see **Extended Data Table 3** for primer sequences), we detected SADS-CoV in acutely sick piglets and sows, but not in recovered or healthy pigs on the four farms, nor in nearby farms without evidence of SADS. The virus replicated to higher titers in piglets than in sows (**Fig. 1B**). SADS-CoV displayed tissue tropism for small intestine (**Fig. 1C**), as observed for other swine enteric coronaviruses[20] and HKU2 in bats[19]. Retrospective PCR analysis revealed that SADS-CoV was present on Farm A during the PEDV epidemic, where the first strongly positive SADS-CoV sample was detected on 6 December 2016. From mid-January onwards, SADS-CoV was the dominant viral agent detected in diseased animals (**Extended Data Figure 2**). Although PEDV was also detected occasionally during the outbreaks in Farms B, C and D, SADS-CoV was the dominant virus (**Extended Data Figure 2 & Table 1**).

We rapidly developed an antibody assay based on the S1 domain of the spike protein using the Luciferase Immunoprecipitation System (LIPS)[21]. As SADS is acute

with rapid onset in piglets, serological investigation was conducted only in sows. Among 46 recovered sows tested, 12 were seropositive for SADS-CoV within three weeks of infection (**Fig. 1D**). To investigate possible zoonotic transmission, serum samples from 35 farm workers who had close contact with sick pigs were subjected to the same LIPS test and none of them was positive for SADS-CoV. Continuous monitoring is required to assess whether the virus has the capacity to mutate and lead to human infection in future.

While the overall genome identity of SADS-CoV and bat CoV HKU2 is 95%, the spike gene (S) sequence identity is only 86%, suggesting that HKU2 is not the direct progenitor of SADS-CoV. To test the hypothesis of a bat origin for SADS-CoV, we developed a qPCR assay based on the SADS-CoV RNA dependent RNA polymerase (RdRp) gene (**Extended Data Table 3**) and screened 596 bat anal swabs collected from 2013-2016 from seven different locations in Guangdong Province (**Extended Data Figure 1**). A total of 71 samples (11.9%) tested positive (**Extended Data Table 4**), almost all of which (94.3%) were from *Rhinolophus* spp. bats (*R. pusillus, R. macrotis, R. sinicus, R. rex and R. affinis*), which are also the natural reservoir hosts of SARS-like coronaviruses[6-8, 22-24]. Complete genome sequences were determined by NGS from four samples that shared highest sequence identity to SADS-CoV, based on the amplicon region (GenBank accession number MF094685−MF094688). These four bat-derived genomes are very similar in size (27.2 kb) to SADS-CoV (**Fig. 2A**) and we tentatively nominate them SADS related coronaviruses (SADSr-CoV). Overall sequence identity to SADS-CoV ranges from

96-98%, higher than the 95% for HKU2-CoV. Importantly, the SADSr-CoV 162140 genome showing highest overall genome identity (98.48%) and S protein sequence identity (98.14%) was sampled in August 2016 less than 100 km from the index farm (**Extended Data Figure 1**). The geographic and temporal alignment of the two events strongly suggests that SADSr-CoV 162140 may be the direct ancestor of SADS-CoV. This is further corroborated by phylogenetic analysis (**Fig. 2B**), that shows bat SADSr-CoVs form a distinct cluster with SADS-CoV in the alpha CoV clade. The major differences among SADSr-CoVs lie in the predicted coding regions of the S and 3'-terminal ORF7a and ORF7b genes (**Fig. 2A**). The S1 domain of the S protein determines CoV host tropism[25]. An additional five S1 genes were sequenced (GenBank accession number MF094697−MF094701), and the S1 of sample 162140 and 141388 were found closest to that of SADS-CoV (**Extended Data Figure 3**). The close relationship among these two viruses and SADS-CoV is further supported by phylogentic analysis of the RdRp gene (**Extended Data Figure 4**).

Known coronavirus host cell receptors include angiotensin-converting enzyme 2 (ACE2) for SARS-related CoV, aminopeptidase N (APN) for PEDV, and dipeptidyl peptidase 4 (DPP4) for MERS-CoV[15,16,25]. To investigate the receptor usage of SADS-CoV, we used SADS-CoV positive samples or HIV pseudoviruses carrying the SADS-CoV S protein to infected HeLa cells which over-expressed all three receptor molecule. While the positive control infected by SL-CoV, MERS-CoV pseudovirus or PEDV showed successful infection or entry, we found no evidence of SADS-CoV

entry, suggesting that none of these three molecules are the functional receptor of SADS-CoV (**Extended Data Table 5**).

Swine enteric coronaviruses including PEDV, transmission gastroenteritis virus (TGEV) and porcine diarrhea coronavirus (PDCoV) are known to cause severe watery diarrhea and dehydration accompanied by histopathological lesions in the infected pigs. Clinically PEDV, TGEV, and PDCoV are indistinguishable[26]. In contrast, piglets infected with SADS-CoV mainly die of nutritional exhaustion rather than severe dehydration. Efforts to isolate virus isolation from intestinal tissues of infected piglets and from bat samples with low PCR Ct values have been unsuccessful to date, so that Koch's postulates cannot be fulfilled using traditional approaches. However, we successfully conducted animal challenge experiments using NGS to identify and confirm causality relationship. Fecal samples positive for SADS-CoV and negative for PEDV or any other known swine diarrhea virus by both NGS and PCR were fed to 3-day or 6-day old piglets. All piglets inoculated with SADS-CoV positive fecal matter exhibited severe diarrhea one day after challenge, while control animals remained healthy. On day 4 post infection, the 3-day but not the 6-day group suffered heavy weight loss and showed signs of nutritional exhaustion and became moribund (**Extended Data Table 6 & Figure 5**). Animals were euthanized for further analysis. Histopathological examinations showed similar lesions in the challenged piglets to those in naturally infected piglets (**Fig. 3A and 3B**). Using rabbit anti-recombinant SADSr-CoV NP serum, specific staining was detected mainly in the small intestines (**Fig. 3C and 3D**). Finally, qPCR and NGS were used to verify that

all diseased piglets were SADS-CoV positive and negative for other known swine diarrhea viruses; and that all control piglets were negative for SADS-CoV. It should be noted that piglets were fed with artificial formula during experimental challenge and the stable nutrient supply mitigated death in most of these animals. Conversely, naturally infected piglets often relied upon poor quantity and quality of milk from infected sows for their nutrition.

The rapid emergence and spread of SADS-CoV, and its high mortality rate in piglets constitute a major economic threat to the pork industry. Viral coinfection is rather common in swine, likely due to intensive farming practices. This was also true on the index farm where co-infection with PEDV and SADS-CoV was detected at the beginning of the outbreak, with SADS-CoV dominant towards later stages of the outbreak. As the barrier for the initial spillover of bat viruses into non-bat hosts is thought to be very difficult to overcome[27], the potential facilitating role of PEDV infection in the emergence of SADS-CoV should be further investigated, especially in the context of known antibody-dependent enhancement of CoV infections[28].

Although bats have been associated with many deadly disease outbreaks impacting both human and livestock, tracing the virus origin usually takes years (for Hendra, Nipah and SARS) if not decades (for Ebola and Marburg). To our knowledge this is the first example where a novel etiological agent discovered during a disease outbreak has been linked with a closely related progenitor virus in bats during the disease investigation itself. Two possible routes of transmission from bats to pigs are plausible: direct transmission via bat fecal contamination of a pig feedlot, and indirect

transmission via an amplifying host, as was originally proposed for SARS-CoV via civets[29]. Further investigation is needed to test these alternative hypotheses once virus isolation is successful.

The current study highlights the value of targeted surveillance in response to an emerging infectious disease event. It also demonstrates that by using modern technological platforms such as NGS and LIPS serology, key experiments that traditionally rely on isolation of live virus could be performed rapidly and prior to virus isolation. Finally, the bat origins of this lethal livestock disease, SARS and most likely MERS demonstrate the disproportionate importance of bats as reservoirs of viruses that threaten veterinary and public health[30].

METHODS

Sample collection

Bats were trapped in their natural habitat in Guangdong Province (**Extended Data Figure 1**). Fecal swab samples were collected in viral transport medium (VTM) composed of Hank's balanced salt solution at pH7.4 containing BSA (1%), amphotericin (15 μg/ml), penicillin G (100 units/ml), and streptomycin (50 μg/ml). Stool samples from sick pigs were collected in VTM. When appropriate and feasible, intestine samples were also taken from deceased animals. Samples were aliquoted and stored at -80 ^{0}C until use. Blood samples were collected from recovered sows and farm workers who had close contact with sick pigs. Serum was separated by centrifugation at 3,000 *g* for 15 min within 24 h of collection and preserved at 4 ^{0}C.

Human serum collection was approved by the Medical Ethics Committee of the Wuhan School of Public Health, Wuhan University and Hummingbird IRB.

Virus isolation

The following cells were used for virus isolation in this study: VeroE6 (cultured in DMEM +10% FBS); *Rhinolophus sinicus* primary or immortalized cells generated by our laboratory (all cultured in DMEM/F12 +15% FBS): kidney primary RsKi9409, lung primary RsLu4323, lung immortalized RsLuT, brain immortalized RsBrT and heart immortalized RsHeT; and swine cell lines: two intestinal IPEC (RPMI1640+10%FBS) and SIEC (DMEM+10%FBS), three kidney PK15, LLC-PK1 (DMEM+10% FBS for the two) and IBRS (MEM+10%FBS), and one testes ST (DMEM+10%FBS).

Cultured cell monolayers were maintained in their respective medium. PCR-positive pig fecal or homogenized pig intestinal supernatant (in 200 μl VTM) were filtered and diluted 1:10 with serum-free medium before being added to cells. After incubation at 37 ^{0}C for 1 h, the inoculum was removed and replaced with fresh culture medium containing 2% FCS. The cells were incubated at 37 ^{0}C and observed daily for cytopathic effect (CPE). Four blind passages (three-day interval between every passage) were performed for each sample. After each passage, both the culture supernatant and cell pellet were examined for presence of virus by RT-PCR using the SADS-CoV primers listed in Table S3. Penicillin (100 units/ml) and streptomycin (15 μg/ml) were included in all tissue culture media.

RNA extraction, S1 gene amplification and qPCR

Whenever commercial kits were used, manufacturer's instructions were followed without modification. RNA was extracted from 200 µl of swab samples (bat), feces or homogenized intestine (pig) with the High Pure Viral RNA Kit (Roche). RNA was eluted in 50 µl of elution buffer and was used as the template for RT-PCR. Reverse transcription was performed using the SuperScript III kit (Invitrogen).

To amplify S1 genes from bat samples, nested PCR was performed with primers designed based on HKU2-CoV (Genbank accession number NC009988.1)[19] (**Extended Data Table 3**). The 25-µl first-round PCR mixture contained 2.5 µl 10X PCR reaction buffer, 5 pmol of each primer, 50 mM MgCl2, 0.5 mM dNTP, 0.1 µl Platinum Taq Enzyme (Invitrogen) and 1 µl cDNA. The 50-µl second-round PCR mixture was identical to the first-round PCR mixture except the primers. Amplification of both rounds was performed as follows: 94 °C for 5 min followed by 60 cycles consisting of 94 °C for 30 s, 50 °C for 40 s, 72 °C for 2.5 min, and a final extension of 72 °C for 10 min. PCR products were gel purified and sequenced.

For qPCR analysis, primers based on SADS-CoV RdRp and NP genes were used (**Extended Data Table 3**). RNA extracted from above was reverse-transcribed using PrimeScript RT Master Mix (Takara). The 10-µl qPCR reaction mix contained 5 µl 2× SYBR premix Ex Taq II (Takara), 0.4 µM of each primer and 1 µl cDNA.

Amplification was performed as follows: 95 °C for 30 s followed by 40 cycles consisting of 95 °C for 5 s, 60 °C for 30 s, and a melting curve step.

Luciferase Immunoprecipitation System (LIPS) assay

LIPS was used in this study due to its simplicity and capacity to be rapidly deployed. The SADS-CoV S1 gene was codon optimized for eukaryotic expression and synthesized (GenScript) in frame with the Renilla luciferase gene (Rluc) and a FLAG tag in the pREN2 vector[21]. pREN2-S1 plasmids were transfected into Cos-1 cells using Lipofectamine (Invitrogen). At 48 h post-transfection, cells were harvested, lysed and a luciferease assay was performed to determine Rluc expression for both the empty vector (pREN2) and the pREN2-S1 construct. For testing of unknown pig or human serum samples, 1 µl of serum was incubated with 10 million units of Rluc alone (vector) and Rluc-S1, respectively, together with 3.5 µl of a 30% protein A/G ultralink beads suspension (Thermo Scientific). After extensive washing to remove unbounded luciferase-tagged antigen, captured luciferease amount was determined using the commercial luciferase substrate kit (Promega). The ratio of Rluc-S1/Rluc(Vector) was used to determine the specific S1 reactivity of pig and human sera. Commercial FLAG antibody (Life Technologies) was used as the positive control, and various pig sera (from uninfected animals in China or Singapore; or pigs infected with PEDV, TGEV or Nipah virus) were used as a negative control.

Protein expression and antibody production

The NP gene from SADSr-CoV 3755 (GenBank accession number MF094702), which shared a 98% aa sequence identity to the SADS-CoV NP gene, was inserted into pET-28a+ (Novagen) for prokaryotic expression. Transformed *E. coli* were grown at 37 °C for 12-18 h in media containing 1 mM IPTG. Bacteria were collected by centrifugation and resuspended in 30 ml of 5 mM imidazole and lysed by sonication. The lysate, from which NP protein expression was confirmed with an anti-HIS-tag antibody, was applied to the Ni2+ resin (Thermo Scientific). The purified NP protein, at a concentration of 400 μg/ml, was used to immunize rabbits for antibody production following published methods[31]. After immunization and two boosts with N protein, rabbits were euthanized and sera were collected. Rabbit anti-N sera were diluted 1:10,000 for subsequent Western blots.

Amplification, cloning and expression of the human and swine genes

Construction of expression clones for human ACE2 in pcDNA3.1 has been described previously[8]. Human DPP4 was amplified from human cell lines. Human APN gene was synthesized. Swine APN and ACE2 genes were amplified from piglet intestine. Full-length gene fragments were amplified using specific primers (provided upon request). The human APN, DPP4 and ACE2 genes were cloned into pCDNA3.1 fused with HIS tag. The pig APN and ACE2 genes were cloned into pCAGGS fused with S tag. Purified plasmids were transfected to HeLa cells. After 24 h, HeLa cells expressing human or swine genes were confirmed by immunofluorescence assay (IFA). Human APN, ACE2 and DPP4 expression was detected using mouse anti-HIS

tag monoclonal antibody or rabbit anti-human APN polyclonal antibody (made by ourselves) followed by cyanin 3-labeled goat anti-mouse/rabbit IgG from proteintech (Proteintech Group). Swine APN and ACE2 expression was detected using mouse anti-S tag monoclonal antibody followed by cyanin 3-labeled goat anti-mouse IgG from proteintech (Proteintech Group).

Pseudovirus preparation

The codon-humanized S protein genes of SADS-CoV and MERS-CoV cloned into pcDNA3.1(+) and pHIV-Luc (pNL4.3.Luc.R$^-$E$^-$Luc) were used for pseudovirus construction as described previously[8,32]. Briefly, 15 μg of each pHIV-Luc (pNL4.3.Luc.R$^-$E$^-$Luc) and the S protein expressing plasmids (or empty vector control) were co-transfected into 4 x 10^6 293T cells using Lipo3000 (Invitrogen) transfection system. After 4 h, the medium was replaced with fresh medium. Supernatants were harvested at 48 h post transfection and separated from cell debris by centrifugation at 3,000g, then by passing through a 0.45μm filter (Millipore). The filtered supernatants were stored at -80°C in aliquots until use. To evaluate the incorporation of S proteins into the core of HIV virions, pseudoviruses in the supernatant (20 ml) were concentrated by ultracentrifugation through a 20% sucrose cushion (5ml) at 80,000g for 90 min using a SW41 rotor (Beckman). Pelleted pseudoviruses were dissolved in 50μl phosphate-buffered saline (PBS) and examined by electron microscopy (EM).

Pseudovirus infection

HeLa cells transiently expressing APN, ACE2 or DPP4 were prepared by a lipo2000 system (Invitrogen). Pseudoviruses prepared above were added to each 96-well plate seeded with Hela cells at 24 h post transfection of APN, ACE2 or DPP4 expression plasmids. The unabsorbed viruses were replaced with fresh medium at 3 h post infection. The infection was monitored by measuring the luciferase activity conferred by the reporter gene carried by the pseudovirus, using the Luciferase Assay System (Promega) as follows: cells were lysed at 48 h post infection, and 20 μl of the lysates was taken for determining luciferase activity by the addition of 50 μl of luciferase substrate.

SADS-CoV positive samples infection and IFA.

HeLa cells transiently expressing APN, ACE2 or DPP4 were prepared by a lipo2000 system (Invitrogen) in 96-well plate, with mock-transfected cells as controls. SADS-CoV RNA positive samples were used to infect Hela cells at 24h post transfection. The inoculum was removed after 1h absorption and washed twice with PBS and supplemented with medium. PEDV, SARS-like-CoV WIV16 and MERS-CoV HIV-pseudovirus were used as positive control for swine APN, human/swine ACE2 and human DPP4, respectively. At 24 h post infection, cells were washed with PBS and fixed with 4% formaldehyde in PBS (pH 7.4) for 20 min at room temperature. SL-CoV WIV16 replication was detected using rabbit antibody against the SL-CoV Rp3 nucleocapsid protein followed by cyanin 3-conjugated goat

anti-rabbit IgG. PEDV and SADS-CoV replication was detected using rabbit antibody against the HKU2 CoV nucleocapsid protein followed by cyanin 3-conjugated goat anti-rabbit IgG. Nucleus was stained with 4',6'-diamidino-2-phenylindole (DAPI). Staining patterns were examined using the FV1200 confocal microscopy (Olympus). The successful infection of MERS CoV HIV-pseudovirus was indicated by luciferase on 48h post infection.

High throughput sequencing and genome assembly

RNA was extracted from the small intestine of deceased pigs and reverse-transcribed into cDNA as described above. Sequencing libraries were constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer's instructions. High throughput sequencing was performed with Illumina MiSeq sequencer. Low quality reads and short reads were filtered. Clean reads were searched against a viral database with the BLASTN program. PCR amplifications were applied to fill the gaps. Amplicons from the same sample were pooled for library preparation and sequenced with the same methodology as described above. All filtered reads were assembled using CLC Genomic Workbench (ver 9.0). 5'-RACE was performed to determine the 5'-end of the genomes. Genomes were annotated using Clone Manager Professional Suite 8 (Sci-Ed Software).

Phylogenetic analysis

SADS-CoV genome sequences and other representative coronavirus sequences (obtained from GenBank) were aligned using MAFFT (ver 7.221). Phylogenetic analyses with full-length genome, S gene and RNA-dependent RNA polymerase gene (RdRp) were performed using MrBayes v3.2 (Stop Valve=0.01) with GTR+G+I model (General Time Reversible model of nucleotide substitution with a proportion of invariant sites and γ-distributed rates among sites).

Animal infection study

Experiments were carried out strictly in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The use of animals in this study was approved by the South China Agricultural University Committee of Animal Experiments (approval ID: 201004152).

Two animal challenge experiments were performed (see detailed planning in **Extended Data Table 6**). Healthy, swine diarrhea virus free, piglets (3- or 6-day old) were orally fed with homogenized intestinal samples from SADS-CoV infected piglets. Inocula were confirmed as SADS-CoV positive, but negative for all other known swine diarrhea viruses. Two control groups of piglets were fed with homogenized intestine from healthy piglets or milk only. Animals were observed daily for signs of disease, such as diarrhea, weight loss and nutritional exhaustion. Fecal swabs were collected daily from all animals and screened for all known swine diarrhea viruses. At experimental endpoints, piglets were humanely euthanized and

402 necropsies performed. Ileal, jejunal and duodenal tissues were taken from selected
403 animals and store in at -80 ^{0}C for further analysis.
404
405 **Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) analysis**
406 Frozen (-80 ^{0}C) small intestinal tissues including duodenum, jejunum, and
407 ileum taken from the above experimentally infected pigs were pre-frozen at -20 ^{0}C for
408 10 min. Tissues were then embedded in optimal cutting temperature compound and
409 cut into 8-μm sections using the Cryotome FSE machine (Thermo Scientific).
410 Mounted microscope slides were fixed with paraformaldehyde and stained with H&E
411 for histopathological examination.
412 For IHC analysis, the rabbit antibody raised above was used for specific
413 staining of SADS-CoV antigen. Slides were blocked by incubating with 10% goat
414 serum (Beyotime) at 37 ^{0}C for 30 min, followed by overnight incubation at 4 ^{0}C with
415 the rabbit anti-3755 N protein serum diluted at 1:1000 in PBST buffer containing 1%
416 goat serum. After washing, slides were then incubated for 50 min at room temperature
417 with HRP conjugated protein A+G (Thermo Scientific) diluted at 1:1000 in PBST
418 buffer containing 1% goat serum. Slides were developed using 3,3' diaminobenzidine
419 substrate (Servicebio) before images were taken using the Pannoramic MIDI system
420 (3D HISTECH).
421
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ACKNOWLEDGEMENTS: We thank Prof. Shao-Bo Xiao for kingly providing pig cell lines, Dr. Peter Burbelo from NIH for kindly providing the LIPS vector and Dr. Li Zhu from GenScript for great facilitation in rapid synthesis of the S gene. We thank the WIV animal facilities and Ms Juan Min for help in preparing IHC samples, and Guangjian Zhu and Aleksei A. Chmura for assistance in bat sampling. This work was jointly supported by China Natural Science Foundation (Y605271JG1 to PZ, 81290341 and 31621061 to ZLS, 31672564 and 31472217 to JYM, 81572045,

81672001 and 81621005 to TYG), National Key Research and Development Program of China (2015AA020108, 2016YFC1202705, SKLPBS1518, AWS16J020 and AWS15J006) to TYG; National Science and Technology Spark Program (2012GA780026) and Guangdong Province Agricultural Industry Technology System Project (2016LM1112) to JYM, State Key Laboratory of Pathogen and Biosecurity (SKLPBS1518) to TYG, Funds for Environment Construction & Capacity Building of GDAS' Research Platform (2016GDASPT-0215) to LBZ, NRF-CRP grant NRF2012NRF-CRP001–056 and CD-PHRG grant CDPHRG/0006/2014 to L-FW, United States Agency for International Development Emerging Pandemic Threats PREDICT project (AID-OAA-A-14-00102), National Institute of Allergy and Infectious Diseases of the National Institutes of Health (Award Number R01AI110964) to PD and ZLS and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDPB0301) to ZLS.

AUTHOR CONTRIBUTIONS: L.F.W, Z.L.S, P.Z, T.Y.G, M.J.Y conceived the study. P.Z, W.Z, Y.Z, M.S, X.S.Z, B.L, X.L.Y, H.G, D.S, Y.L, X.L.L, J.C performed qPCR, serology, histology and virus culturing. H.F, Y.W.Z, J.M.L, G.Q.P, X.P.A, Z.Q.M, T.T.H, Y.H, Q.S, X.L.L.Z performed genome sequencing and annotations. T.L, Q.M.X, J.W.C, L.Z, K.J.M, Z.X.W, L.B.Z, S.Y.L, Y.S,C, Y.S prepared the samples and animal challenges. Z.L.S., P.D., L.B.Z, S.Y.L coordinated collection of bat samples. P.Z, L.F.W, Z.L.S, P.D prepared the draft.

AUTHOR INFORMATION

Full-length genomic sequences or S sequences of SADS-CoV and SARSr-CoV have been deposited in GenBank under accession numbers MF094681-MF094688 and MF094697-MF094701, respectively.

The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to ZLS. (zlshi@wh.iov.cn).

FIGURE LEGENDS

Figure 1. Detection of SADS-CoV infection in pigs in Guangdong, China.

(A) Chronology of outbreaks and the mortality rate on the four different farms. Daily number of pig deaths was recorded from 26 October 2016 to 2 May 2017. The outbreak is ongoing as of the current date. (B) Detection of SADS-CoV by qPCR in different groups of pigs. (C) Tissue distribution of SADS-CoV in diseased pigs. LA- Lymphonodi abdominals; LM- Lymphoglandulae mesentericae. (D) Detection of SADS-CoV antibodies using S1-specific LIPS assay. Infected sows were bled during the initial three weeks of the outbreak, then >1 month after the beginning of the outbreak. Healthy pig sera were set as control.

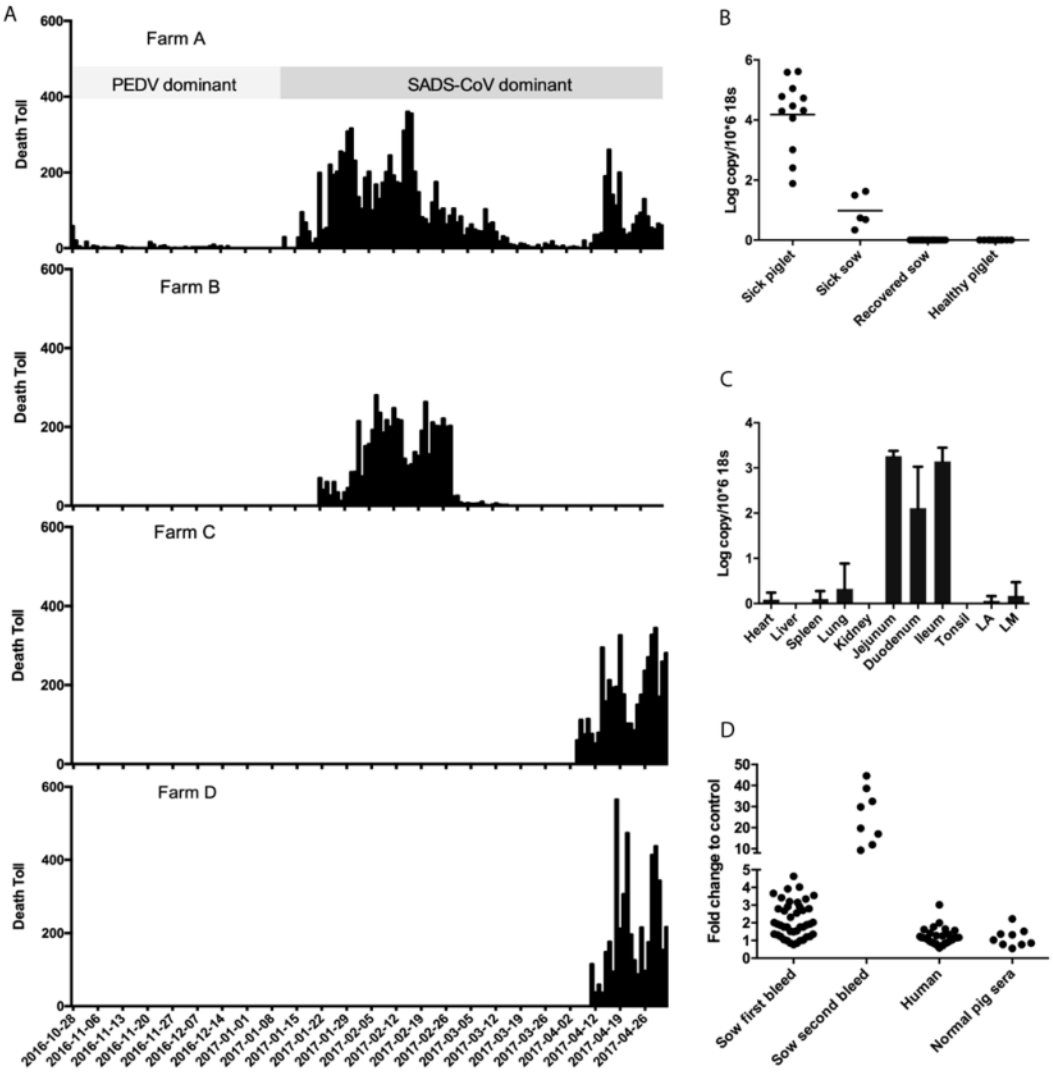


Figure 2. Genome and phylogeny analysis of SADS-CoV and SADSr-CoV.

(A) Genome organization and comparison. Colored boxes represent genes or UTRs: Green- nonstructural polyproteins ORF1a and 1b; Yellow- structural proteins S, E, M and N; Blue- nonstructural accessory proteins NS3a, NS7a and Ns7b; Orange- UTRs. The level of sequence identity of SADSr-CoV to SADS-CoV was illustrated by patterns; Open box- highly similar; Dots- moderately similar; Dashed line- least similar. (B) Phylogenetic tree based on full-length genome sequences. The Bayesian tree was constructed using MrBayes v3.2 with the average standard deviation of split frequencies under 0.01. The host of each sequence was marked by the animal symbols on the right and newly sequenced SADS-CoVs are highlighted in red while bat SADSr-CoVs in blue.

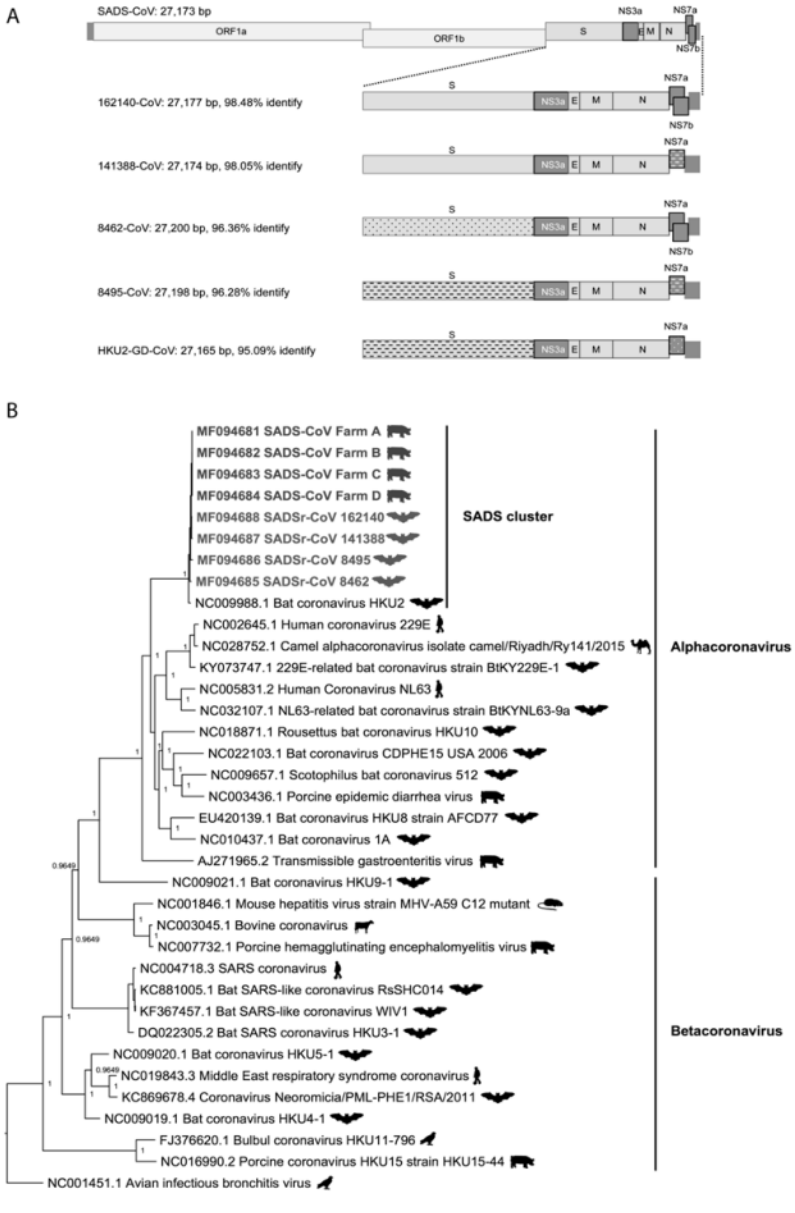
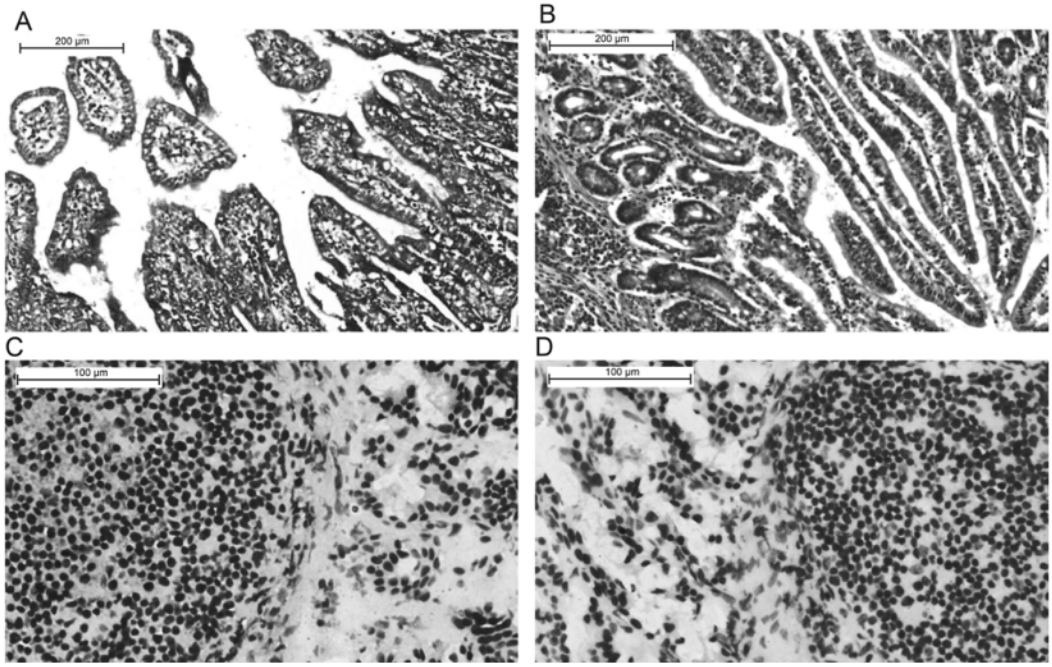


Figure 3 Immunohistopathology of SARS-CoV infected tissues.

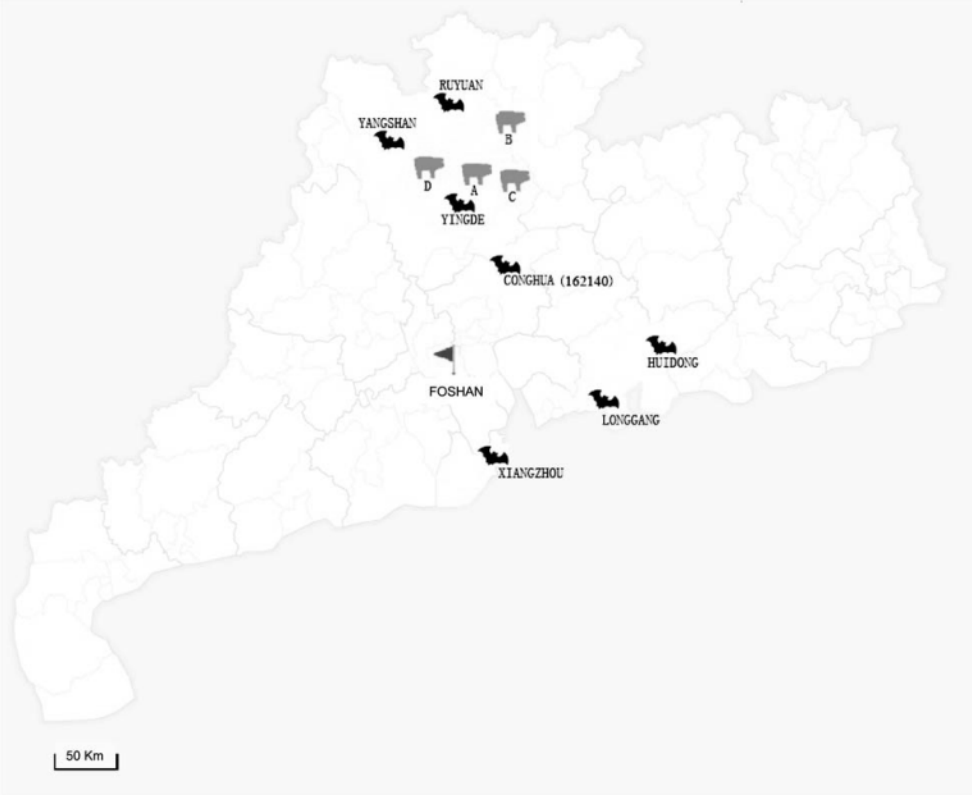
(A) and (B), Hematoxylin and eosin staining of jejunum with and without infection. (C) and (D), Immunohistochemistry staining of jejunum with and without infection using rabbit serum raised against the recombinant SADSr-CoV NP protein.



EXTENDED DATA LEGENDS

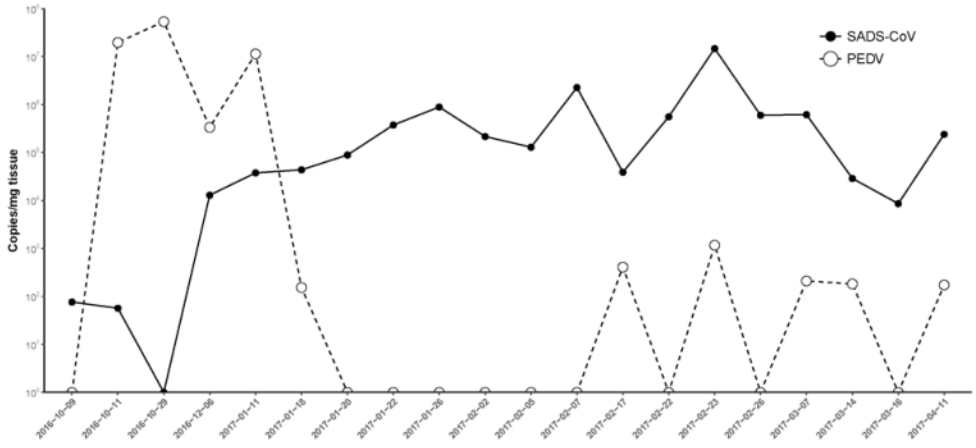
Extended Data Figure 1. Map of Guangdong Province, China.

SADS-affected farms are labeled A to D with blue swine symbols following the temporal sequence of the outbreaks. Bat sampling sites are identified by black bat symbols. The bat SADSr-CoV most closely related to SADS-CoV (sample 162140) originated Conghua. The red flag marks Foshan city, site of the index case of SARS..



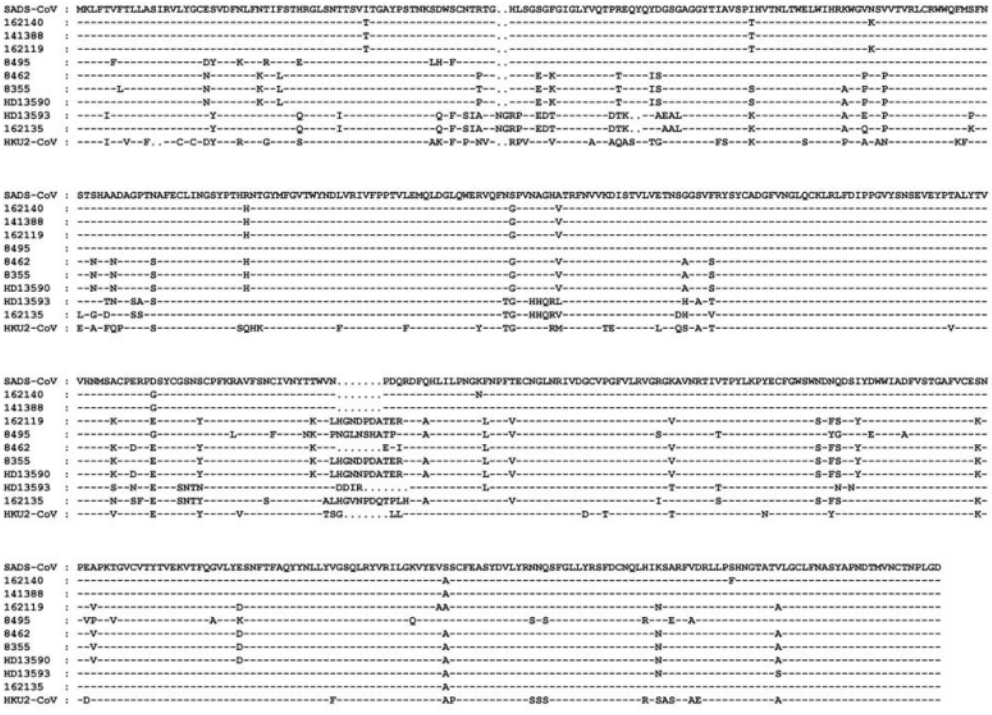
Extended Data Figure 2. Co-circulation of PEDV and SADS-CoV during the initial outbreak on Farm A.

Pooled intestinal samples were collected at dates given on the x-axis from deceased piglets and analyzed by qPCR. The intensity infection for each piglet is shown as a copy number per milligram of intestine (y-axis).



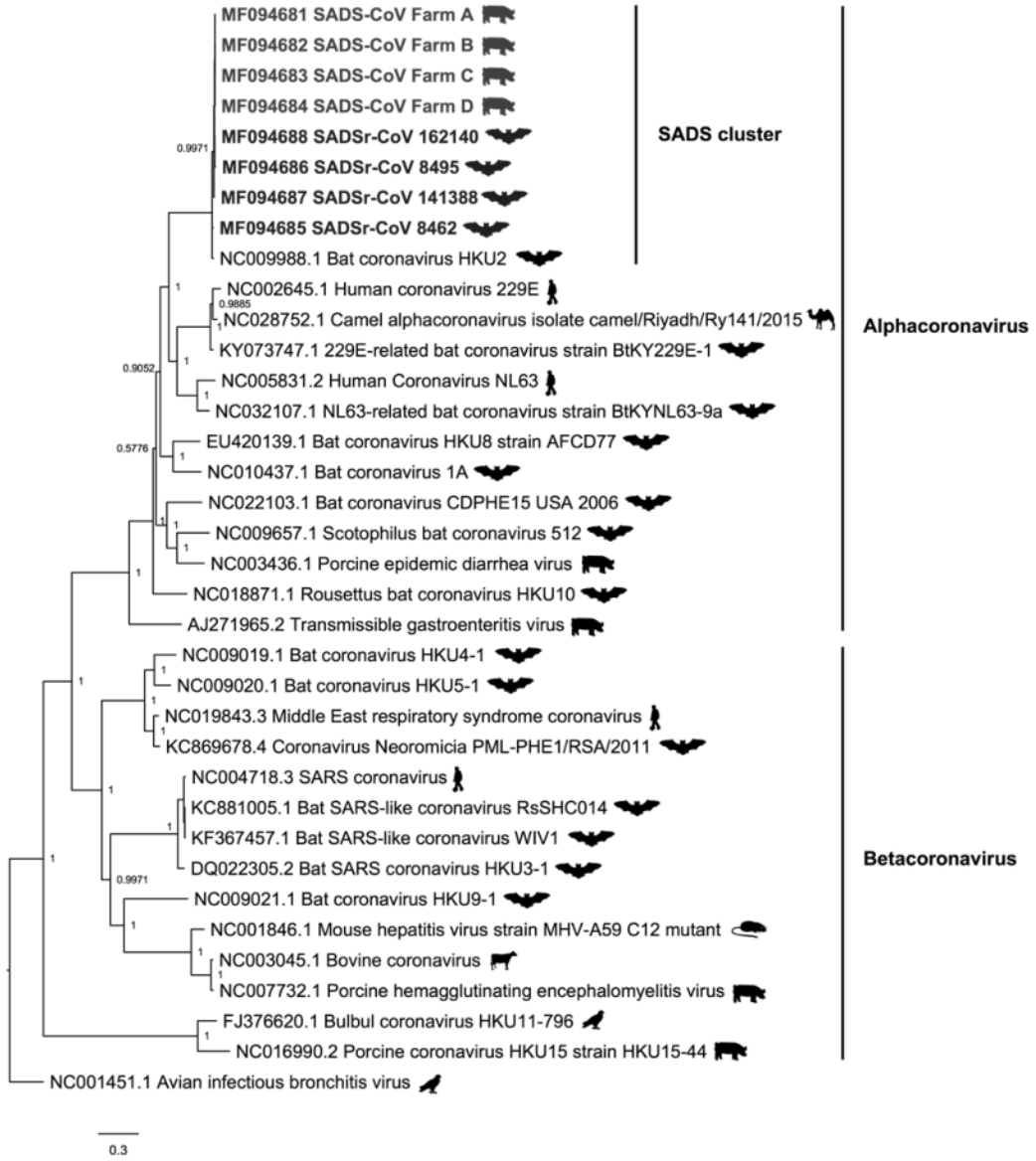
Extended Data Figure 3. Alignment of amino acid sequences of S1 proteins of the SADS cluster of coronaviruses.

The top sequence is from SADS-CoV Farm A. The four SADSr-CoV S1 sequences (162140, 141388, 162119 and 162135) were derived from NGS whole genome sequencing. HKU2-CoV is from a published report[19]. Five additional S1 sequences (8495, 8462, 8355, HD13590 and HD13593) from bats were determined by PCR and Sanger sequencing as described in the text. Dashed lines indicate identical residues while dots represent gaps.



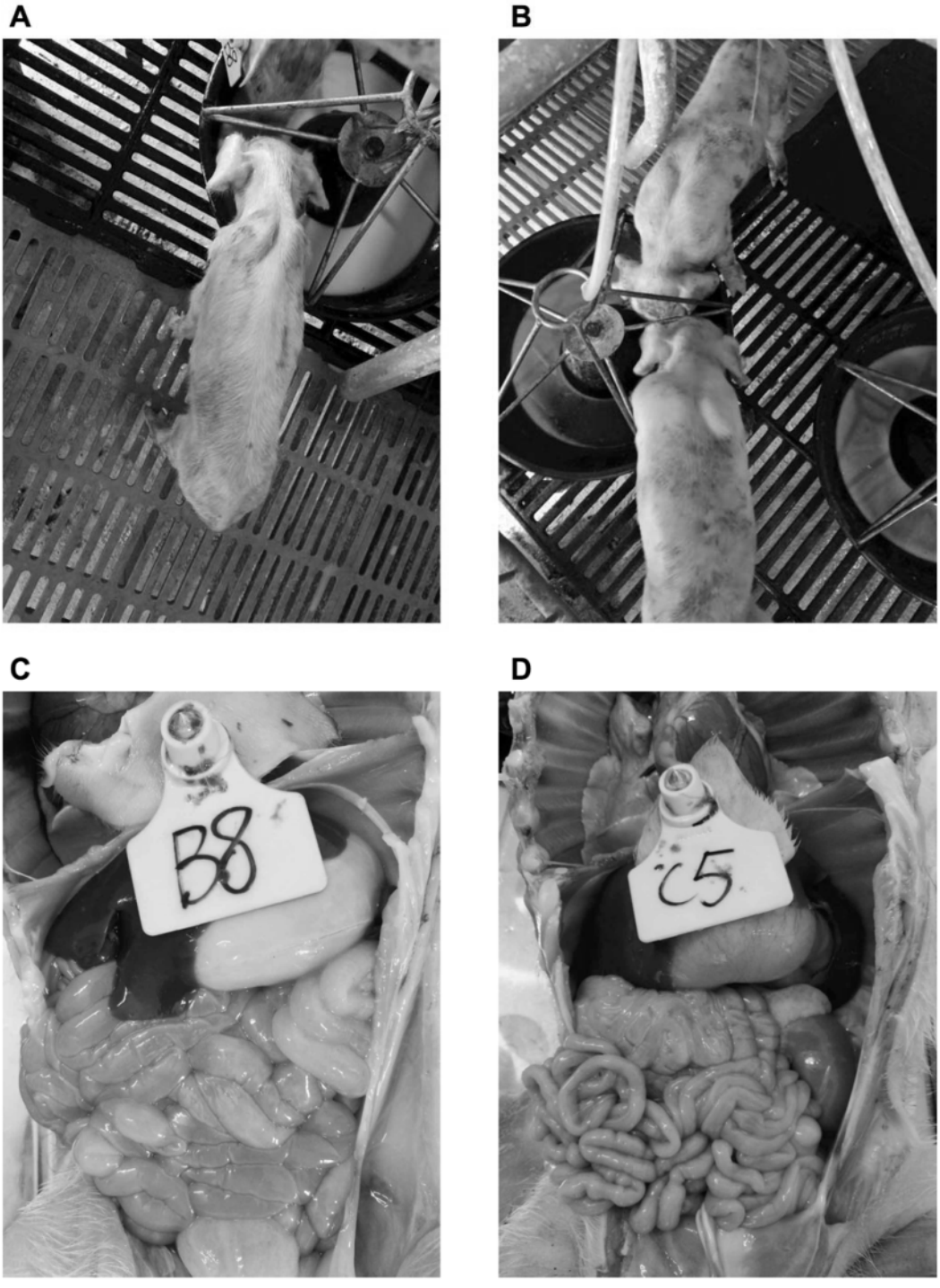
Extended Data Figure 4. Bayesian phylogenetic tree of the sequences encoding RdRp of SADS-CoV and related coronaviruses.

Tree was constructed using MrBayes v3.2 with the average standard deviation of split frequencies under 0.01. The host of each sequence is represented pictorially. Newly sequenced SADS-CoVs are highlighted in red while bat SADSr-CoVs are highlighted in blue.



Extended Data Figure 5. SADS-CoV experimentally infected and healthy piglets.

(A) Piglet on day 2 post SADS-CoV infection. (B) Mock infected piglet on day 2. (C) Intestine from infected piglet at necropsy. (D) Intestine from mock-infected piglet at necropsy.



Extended Data Table 1. List of all known swine viruses tested by PCR at the beginning of the of SADS outbreak investigation on the four farms *.

	PEDV	PDCoV	TGEV	RV	PBV	PSV	SVA	SIV	NADC30	PRRV	FMDV	CSFV	PCV2	PCV3	APPV	PPV	Norovirus
Farm A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-
Farm B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-
Farm C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND
Farm D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND

* Dash indicates negative PCR result. ND, not done. Virus abbreviations: PEDV- Porcine Epidemic Diarrhea Virus; PDCoV- Porcine Delta Coronavirus; TGEV-Porcine Transmissible Gastroenteritis Virus; RV- Porcine Rotavirus; PBV- Porcine Picobirnavirus; PSV- Porcine Sapelo Virus; SVA- Porcine Senecavirus A; SIV- Swine Influenza Virus; PPRV- Porcine Reproductive and Respiratory Syndrome Virus, strain NADC30; PRV- Porcine Pseudorabies Virus; FMDV- Foot and Mouth Disease Virus; CSFV- Classical Swine Fever Virus; PCV2- Porcine Circovirus 2; PCV3- Porcine Circovirus 3; APPV- Atypical Porcine Pestivirus; PPV- Porcine Parvovirus.

Extended Data Table 2. List of nucleotide and amino acid (aa) residue variants among the SADS-CoV genomes obtained from the four different farms.

Nucleotide residue position*	Gene name	Amino acid (aa) residue position*	Farm A nucleotide (aa)	Farm B nucleotide (aa)	Farm C nucleotide (aa)	Farm D nucleotide (aa)
2236	ORF1a	645	G(A)	G(A)	G(A)	T(S)
2955	ORF1a	884	T(G)	C(G)	T(G)	T(G)
3285	ORF1a	994	G(E)	G(E)	G(E)	T(D)
15395	ORF1b	915	C(T)	C(T)	C(T)	T(T)
18410	ORF1b	1920	C(G)	T(G)	T(G)	T(G)
20219	ORF1b	2523	C(L)	T(L)	T(L)	T(L)
21622	S	379	C(N)	C(N)	C(N)	A(K)

Non-synonymous aa substitutions are marked in red. * SADS-CoV from Farm A was used as the reference sequence, from which the residue numbering was derived.

627 **Extended Data Table 3. List of PCR primers used in this study.**

628

Gene	Primer name and location*	Primer sequence	Application
RdRp gene	SADS-RdRp-F (19512-19531) SADS-RdRp-R (19590-19608)	GTTGATTGTAAGGCTTGGCG AACCACACTTCCACTCAGC	qPCR
N gene	SADS-N-F (25810-25830) SADS-N-R (25938-25957)	CTAAAACTAGCCCCACAGGTC TGATTGCGAGAACGAGACTG	qPCR
S gene	HKU2-S1-1F (20066-20085) HKU2-S1-1R (22317-22336)	GGCGCTATGGCTGTTAAGAT CACGAATGTCAGCCTCAACT	Cloning
S gene	HKU2-S1-2F (20157-20176) HKU2-S1-2R (22218-22238)	CCAGTGTCAACACGTCATCT ACGCTGAACTTAGGCATTGTA	Cloning

629 * The numbering system of SADS-CoV from Farm A was used as for Extended Data Table 2.

630

Extended Data Table 4. List of SADSr-CoVs detected in bats in Guangdong, China.

Sampling			PCR analysis	
Time (Month-Year)	Location	Bat Species	Fecal swabs sampled	PCR Positive
Jun 13	Yingde	*Rhinolophus sinicus*	1	1
		Pipistrellus abramus	8	0
		Myotis ricketti	2	0
Jul 13	Yangshan	*Pipistrellus abramus*	1	0
		Hipposideros pratti	36	1
Jul 13; May 14; Jun 15; Aug 16	Ruyuan	*Rhinolophus sinicus*	27	6
		Rhinolophus affinis	11	2
		Rhinolophus macrotis	3	0
		Rhinolophus pusillus	41	6
		Rhinolophus rex	9	7
		Hipposideros pratti	7	0
Sep 14; Jun 15; Aug 16	Conghua	*Rhinolophus sinicus*	70	2
		Rhinolophus affinis	34	7
		Rhinolophus pusillus	11	2
		Hipposideros pomona	10	0
		Myotis ricketti	1	0
Jun 13; Nov 13; Aug 14; Jun 15	Huidong	*Rhinolophus sinicus*	37	2
		Rhinolophus affinis	59	29
		Rhinolophus macrotis	15	2
		Rhinolophus pusillus	1	0
		Hipposideros pomona	2	0
		Myotis ricketti	84	1
Apr 14; Jun 15	Longgang	*Rhinolophus sinicus*	55	1
		Pipistrellus abramus	5	1
Sep 14	Xiangzhou	*Rhinolophus pusillus*	28	0
		Hipposideros pomona	38	1
		Total	596	71 (11.9%)

See Fig. S1 for sampling sites in relation to SARS and SADS outbreak locations

Extended Data Table 5. Multiple human CoV receptors as well as swine APN cannot be utilized as entry receptor for SADS-CoV.

	HuAPN★	HuACE2★	HuDPP4★	SwAPN★	SwACE2★
SADS-CoV*	-	-	-	-	-
SARS-like-CoV	NA	+	NA	NA	+
MERS-CoV#	NA	NA	+	NA	NA
PEDV	NA	NA	NA	NA	NA
Expression$	+ (APN Ab)	+ (HIS-tag Ab)	+ (DPP4 Ab)	+ (S-tag Ab)	+ (S-tag Ab)

★Gene accession numbers for the genes used in this study: human APN, M22324.1; human ACE2, NM_021804; human DPP4, NM_001935.3; swine APN, NM_214277.1; swine ACE2, XM_021079374.1

* For SADS-CoV infection, both positive samples and HIV-pseudovirus were used. Viral positive samples were from SADS infected pig anal swabs: SusAS-7 (4.0×10^5 copy/μl), SusAS-20 (4.3×10^5 copy/μl), SusAS-22 (2.4×10^5 copy/μl).

For MERS-CoV infection, HIV-pseudovirus were used.

$ Expression of APN, DPP4 and ACE2 was confirmed by antibodies against the targeting proteins or fused tags.

Extended Data Table 6. Experimental outline of SADS-CoV infection of piglets.

Experiments were performed with (A) 3-day old or (B) 6-day old piglets. Infection was performed as described in the Material and Methods.

A

Groups	Infection material	Number	Age (days)	Infection Dose	Infection route	SADS-CoV titer (copy/μl)	First day				Second day				Fourth day
							Severe diarrhea	Weight loss	SADS-CoV postive	PEDV/PDCoV/RV positive	Severe diarrhea	Weight loss	SADS-CoV postive	PEDV/PDCoV/RV positive	Nutrition exhaustion and Dying
A	SC1 (SADS-CoV positive)	5	3	3mL	Oral+milk	6.54×10^5	5	5	5	0	5	5	5	0	3
B	DE2 (SADS-CoV positive)	5	3	3mL	Oral+milk	10.62×10^5	5	4	5	0	5	3	5	0	1
C	Mock	4	3	3mL	Oral+milk	0	0	0	0	0	0	0	0	0	0
D	Empty mock	4	3	0ml	Milk only	0	3 mild diarrhea	0	0	0	1	0	0	0	0

B

Groups	Infection material	Number	Age (days)	Infection Dose	Infection route	SADS-CoV titer (copy/mg)	First day				Second day				Fourth day
							Severe diarrhea	Weight loss	SADS-CoV postive	PEDV/PDCoV/RV positive	Severe diarrhea	Weight loss	SADS-CoV postive	PEDV/PDCoV/RV positive	Nutrition exhaustion and Dying
A	SC1 (SADS-CoV positive)	6	6	2mL	Oral+milk	6.54×10^5	5	3	6	0	1	0	6	0	0
B	DE2 (SADS-CoV positive)	5	6	2mL	Oral+milk	1.20×10^5	3	3	5	0	3 moderate	1	5	0	0
C	Mock	6	6	2mL	Oral+milk	0	0	0	0	0	0	0	0	0	0
D	Empty mock	5	6	0ml	Milk only	0	0	0	0	0	0	0	0	0	0

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thu, 29 Jun 2017 11:17:15 +0000
To: Peter Daszak; Hongying Li
Cc: Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Thanks Peter. I'll have this on standby. Looking forward to seeing you shortly.

Erik

From: Peter Daszak (b)(6)
Sent: Thursday, June 29, 2017 12:43 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6) Hongying Li (b)(6)
Cc: Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Erik,

In case NIAID has issues with USB drives etc., here is a pdf version of our talk for tomorrow morning. I hope you can have that as a backup from your email in case we can't download our talk from our laptops.

Look forward to seeing you.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. (b)(6)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, June 26, 2017 9:30 AM
To: Hongying Li
Cc: Peter Daszak; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Thank you Hongying. I will forward it to security. Looking forward to your visit later this week.

Erik

From: Hongying Li (b)(6)
Sent: Monday, June 26, 2017 9:25 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Peter Daszak (b)(6) Aleksei Chmura (b)(6)
Alison Andre (b)(6)
Subject: Re: Potential visit to NIH by our Chinese Co-investigator in June?

Dear Erik,

Not sure if this is too late, but wanted to send you the updated attendee information with Peng Zhou's visa number. Please find it in the attachment. Let me know if there is any question.

Thanks,
Hongying

On Jun 16, 2017, at 11:22 AM, Hongying Li (b)(6) wrote:

Dear Erik,

Please find the security screening information for Zhengli Shi, Peng Zhou, and Hongying Li in the attachment. We don't have the visa No. for Peng Zhou at this moment because his visa application is still under administrative processing at the Embassy. We are not sure if he can obtain his visa on time or not, but will let you know as soon as we have any further confirmed information.

Please let me know if there is any question. Thank you!

Best,
Hongying

<5601 Foreign Visitor Form-China.xlsx>
On May 24, 2017, at 3:16 PM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Peter,
Thanks for this information. I've attached a form that will help expedite security screening for Dr Zhou and Hongying Li. Can you please have them complete the information on the second sheet of the attachment? I'll need to turn it in to our security office at least a week before your visit, so if you could get it back to me by June 19th or 20th that would be great. Also, please let them know they should bring their passports with them. Everyone else will need a photo ID as well.

Let me know if you need directions to our building. I would suggest planning to arrive between 8:15 and 8:30, as there can be a line at security if there are other public meetings occurring that day. There is no visitor parking at our facilities, but there is a public parking garage on our block that I can get validation stickers for if you'll be driving. We are also a short walk from the Twinbrook Metro stop, if you plan to travel by train.

Erik

From: Peter Daszak (b)(6)
Sent: Wednesday, May 24, 2017 3:05 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?
Importance: High

Hi Erik,

Great to hear from you and looking forward to the talk on June 29th

We're proposing for 4 people to visit NIAID and I've attached bios for all of them to this email. Note that Dr Shi, Dr. Zhou and Hongying Li are all Chinese nationals, and I'm not sure what sort of clearance you'll need for that, so please let me know and we'll work on getting the relevant documents to you

1. Myself, PI on the NIAID CoV grant, President of EcoHealth Alliance, EHA lead on the USAID PREDICT project
2. Dr. Zhengli Shi, Co-Investigator on the NIAID CoV grant, Director of Center for Emerging Diseases at The Wuhan Institute of Virology
3. Dr. Peng Zhou, Associate Professor at Wuhan Institute of Virology
4. Hongying Li, Research Scientist and Country Liaison for China at EcoHealth Alliance

Re a title for the talk, bearing in mind it should be broader than just SARS-CoV, what about the following:

"SARS, MERS and the risk of novel viral emergence from bats"

Zhengli and I will do a double act, and we'll cover the work we're doing on the NIAID project, as well as the broadscale surveillance of bats for novel viruses in PREDICT.

Cheers,

Peter

Peter Daszak
President

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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Stemmy, Erik (NIH/NIAID) [E] [mailto:erik.stemmy@nih.gov]
Sent: Thursday, May 18, 2017 8:26 AM
To: Peter Daszak
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Peter,
We've got you on the calendar for June 29th. Can you send me a title for the talk, short summary, and brief bios for the presenters?

Thank you!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Monday, April 24, 2017 4:47 PM
To: Peter Daszak (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

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Erik

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Sent: Monday, April 24, 2017 4:44 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

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We'll plan to come to DC the afternoon or evening before and then do the symposium and meet with you.

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, April 24, 2017 4:35 PM
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Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

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Subject: Potential visit to NIH by our Chinese Co-investigator in June?
Importance: High

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<5601 Foreign Visitor Form.xlsx>

Hongying Li, MPH 李泓萤
China Programs Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b)(6) (U.S. mobile)
132.6145.1848 (China mobile)
Hongying Li (Skype)
756614210 (WeChat)

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From: Peter Daszak
Sent: Thu, 29 Jun 2017 04:42:34 +0000
To: Stemmy, Erik (NIH/NIAID) [E]; Hongying Li
Cc: Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?
Attachments: Peter Daszak Zhengli Shi NIAID June 2017.pdf

Erik,

In case NIAID has issues with USB drives etc., here is a pdf version of our talk for tomorrow morning. I hope you can have that as a backup from your email in case we can't download our talk from our laptops.

Look forward to seeing you.

Cheers,

Peter

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President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Monday, June 26, 2017 9:30 AM
To: Hongying Li
Cc: Peter Daszak; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Thank you Hongying. I will forward it to security. Looking forward to your visit later this week.

Erik

From: Hongying Li (b)(6)
Sent: Monday, June 26, 2017 9:25 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Peter Daszak (b)(6) Aleksei Chmura (b)(6)
Alison Andre (b)(6)
Subject: Re: Potential visit to NIH by our Chinese Co-investigator in June?

Dear Erik,

Not sure if this is too late, but wanted to send you the updated attendee information with Peng Zhou's visa number. Please find it in the attachment. Let me know if there is any question.

Thanks,
Hongying

On Jun 16, 2017, at 11:22 AM, Hongying Li (b)(6) wrote:

Dear Erik,

Please find the security screening information for Zhengli Shi, Peng Zhou, and Hongying Li in the attachment. We don't have the visa No. for Peng Zhou at this moment because his visa application is still under administrative processing at the Embassy. We are not sure if he can obtain his visa on time or not, but will let you know as soon as we have any further confirmed information.

Please let me know if there is any question. Thank you!

Best,
Hongying

<5601 Foreign Visitor Form-China.xlsx>
On May 24, 2017, at 3:16 PM, Stemmy, Erik (NIH/NIAID) [E] (b)(6) wrote:

Hi Peter,
Thanks for this information. I've attached a form that will help expedite security screening for Dr Zhou and Hongying Li. Can you please have them complete the information on the second sheet of the attachment? I'll need to turn it in to our security office at least a week before your visit, so if you could get it back to me by June 19th or 20th that would be great. Also, please let them know they should bring their passports with them. Everyone else will need a photo ID as well.

Let me know if you need directions to our building. I would suggest planning to arrive between 8:15 and 8:30, as there can be a line at security if there are other public meetings occurring that day. There is no

visitor parking at our facilities, but there is a public parking garage on our block that I can get validation stickers for if you'll be driving. We are also a short walk from the Twinbrook Metro stop, if you plan to travel by train.

Erik

From: Peter Daszak (b)(6)
Sent: Wednesday, May 24, 2017 3:05 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Cc: Hongying Li (b)(6) Aleksei Chmura (b)(6) Alison Andre (b)(6)
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?
Importance: High

Hi Erik,

Great to hear from you and looking forward to the talk on June 29th

We're proposing for 4 people to visit NIAID and I've attached bios for all of them to this email. Note that Dr Shi, Dr. Zhou and Hongying Li are all Chinese nationals, and I'm not sure what sort of clearance you'll need for that, so please let me know and we'll work on getting the relevant documents to you

1. Myself, PI on the NIAID CoV grant, President of EcoHealth Alliance, EHA lead on the USAID PREDICT project
2. Dr. Zhengli Shi, Co-Investigator on the NIAID CoV grant, Director of Center for Emerging Diseases at The Wuhan Institute of Virology
3. Dr. Peng Zhou, Associate Professor at Wuhan Institute of Virology
4. Hongying Li, Research Scientist and Country Liaison for China at EcoHealth Alliance

Re a title for the talk, bearing in mind it should be broader than just SARS-CoV, what about the following:

"SARS, MERS and the risk of novel viral emergence from bats"

Zhengli and I will do a double act, and we'll cover the work we're doing on the NIAID project, as well as the broadscale surveillance of bats for novel viruses in PREDICT.

Cheers,

Peter

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President

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From: Stemmy, Erik (NIH/NIAID) [E] (b)(6)
Sent: Thursday, May 18, 2017 8:26 AM
To: Peter Daszak
Cc: Hongying Li; Aleksei Chmura; Alison Andre
Subject: RE: Potential visit to NIH by our Chinese Co-investigator in June?

Hi Peter,
We've got you on the calendar for June 29th. Can you send me a title for the talk, short summary, and brief bios for the presenters?

Thank you!
Erik

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Sent: Monday, April 24, 2017 4:47 PM
To: Peter Daszak (b)(6)
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EcoHealth Alliance

SARS, MERS and the risk of novel viral emergence from bats

Peter Daszak
EcoHealth Alliance, New York, USA
www.ecohealthalliance.org

Zhengli Shi
Wuhan Institute of Virology, China

Local conservation.
Global health.

NIH 57943 - 003575

New viruses from bat reservoir hosts in the last 20+ years

- 1994 – Hendra virus (horses)
- 1997 – Australian fruit bat lyssavirus (direct)
- 1997 – Menangle virus (pigs)
- 1999 – Nipah virus (pigs)
- 2001-13 – Nipah Bangladesh/India (direct)
- 2003 – SARS-CoV(direct)
- 2006 – Melaka virus
- 2013 - MERS (camels)
- 2017 – SADS-CoV pigs

Ebola, Marburg, African henipaviruses…..

NIH 57943 - 003576

Early SARS cases, Guangdong China

Table 2. SARS cases (%) by month of onset and occupational status, Guangdong,

Occupational status[b]	Jan 2003 or before no. (%)	Feb 2003 (%)	M
Retired	2 (9)	44 (10)	
Worker	2 (9)	40 (9)	
Student	0 (0)	29 (7)	
Civil servant	3 (13)	43 (10)	
Housewife	0 (0)	20 (5)	
Food industry worker	9 (39)	20 (5)	
Farmer	1 (4)	10 (2)	
Teacher	1 (4)	7 (2)	
Child	0 (0)	9 (2)	
Other	2 (9)	49 (11)	
Unknown	3 (13)	157 (37)	
Total	23 (100)	428 (100)	

[a]SARS, severe acute respiratory syndrome.
[b]Excluding healthcare workers or case-patients with known exposure.

Xu *et al.* EID 2004


(b)(6)
(b)(6)

Bats Are Natural Reservoirs of SARS-Like Coronaviruses

Wendong Li,[1,2] Zhengli Shi,[2*] Meng Yu,[3] Wuze Ren,[2] Craig Smith,[4] Jonathan H. Epstein,[5] Hanzhong Wang,[2] Gary Crameri,[3] Zhihong Hu,[2] Huajun Zhang,[2] Jianhong Zhang,[2] Jennifer McEachern,[3] Hume Field,[4] Peter Daszak,[5] Bryan T. Eaton,[3] Shuyi Zhang,[1,6*] Lin-Fa Wang[3*]

Severe acute respiratory syndrome (SARS) emerged in 2002 to 2003 in southern China. The origin of its etiological agent, the SARS coronavirus (SARS-CoV), remains elusive. Here we report that species of bats are a natural host of coronaviruses closely related to those responsible for the SARS outbreak. These viruses, termed SARS-like coronaviruses (SL-CoV), display greater genetic variation than SARS-CoV isolated from humans or from civets. The human and civet isolates of SARS-CoV nestle phylogenetically within the spectrum of SL-CoVs, indicating that the virus responsible for the SARS outbreak was a member of this coronavirus group.

survey bats in the search for the natural reservoir of SARS-CoV.

In this study, conducted from March to December of 2004, we sampled 408 bats representing nine species, six genera, and three families, from four locations in China (Guangdong, Guangxi, Hubei, and Tianjin) after trapping them in their native habitat (Table 1). Blood, fecal, and throat swabs were collected; serum samples and cDNA from fecal or throat samples were independently analyzed, double-blind, with different methods in Wuhan and Geelong (*14*).

Among six genera of bat species surveyed (*Rousettus, Cynopterus, Myotis, Rhinolophus, Nyctalus*, and *Miniopterus*), three communal, cave-dwelling species from the genus *Rhinolophus* (horseshoe bats) in the family *Rhinolophidae* demonstrated a high SARS-CoV antibody prevalence: 13 out of 46 bats (28%) in *R. pearsoni* from Guangxi, 2 out of 6 bats (33%) in *R. pussilus* from Guangxi; and 5 out

Li *et al.* (2005) *Science* 310: 676-679

Are bats special?

- Do bats harbor more zoonoses than other wildlife groups?
- What risk do bat-origin coronaviruses represent?
- Could bats provide strategies to combat lethal viruses?

"a balance of host response and virus replication is essential for establishment of a reservoir host/virus relationship. Thus, it is likely that bats and their viruses have co-adapted in a relationship that limits disease, but also impairs antiviral responses"

Schountz *Viruses* 2014

Database Summary

2805 unique mammal-virus associations

754 mammal species

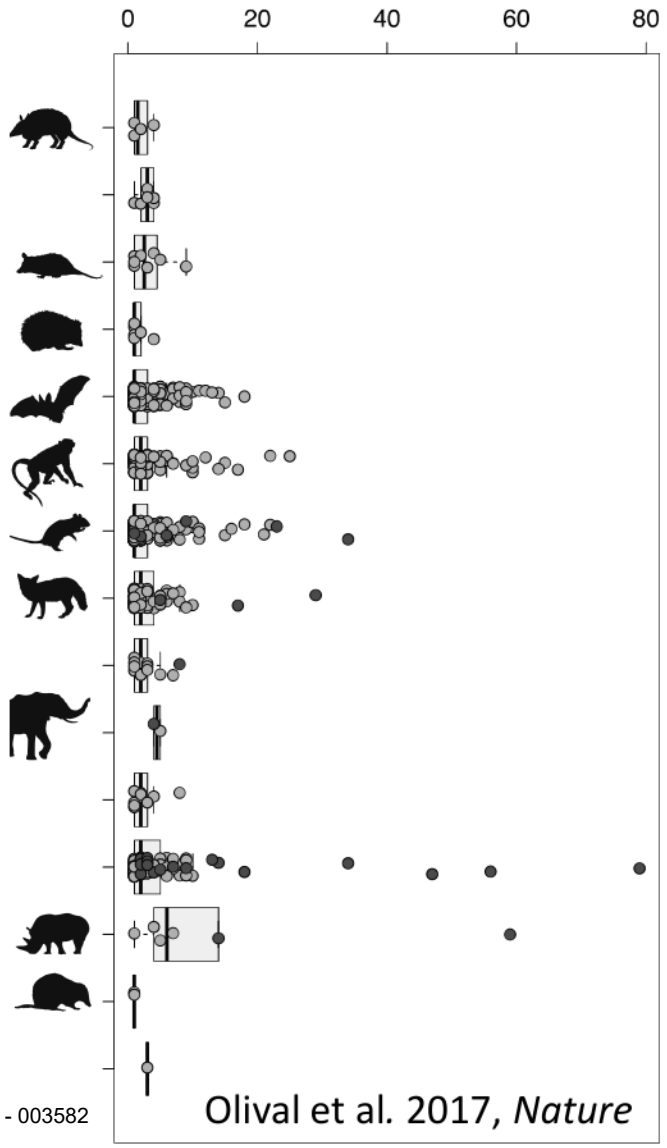
- 374 genera, 80 families, 15 orders

586 ICTV unique viruses found in mammals

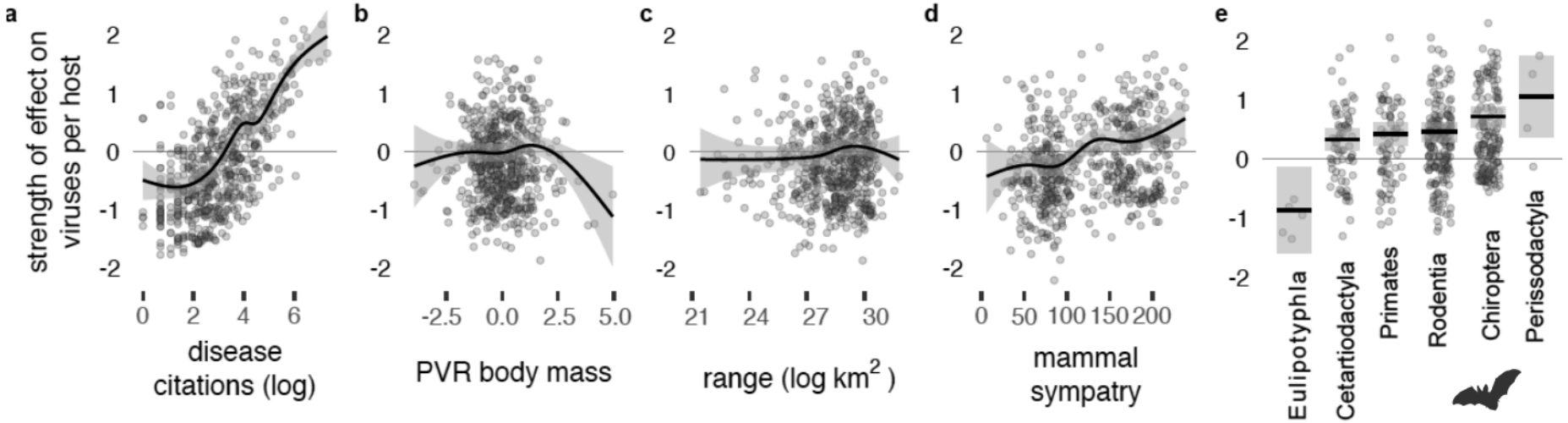
- 28 viral families
- 382 RNA; 205 DNA viruses
- 263 detected in humans (44%); 75 exclusively human.
- **188 (71.5%) of human viruses are 'zoonotic'**

Olival et al. 2017, *Nature*

Observed Viral Richness in Mammals

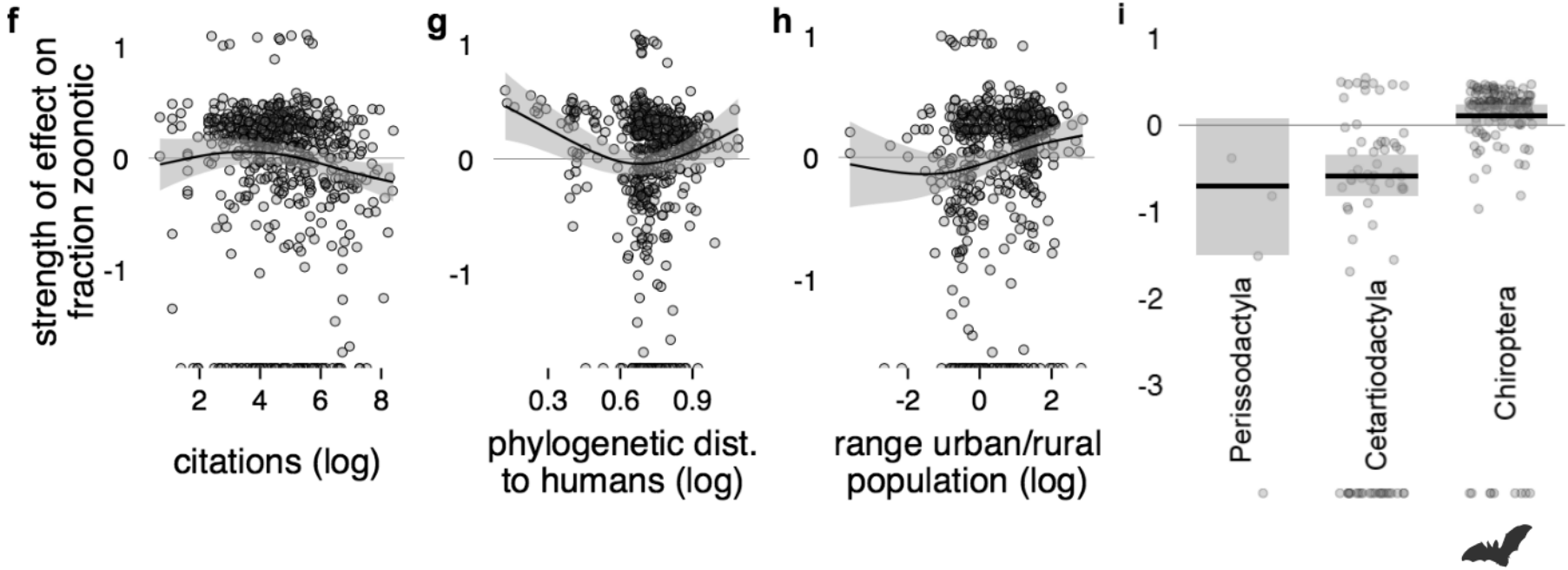


Predictors of total viral richness per spp.



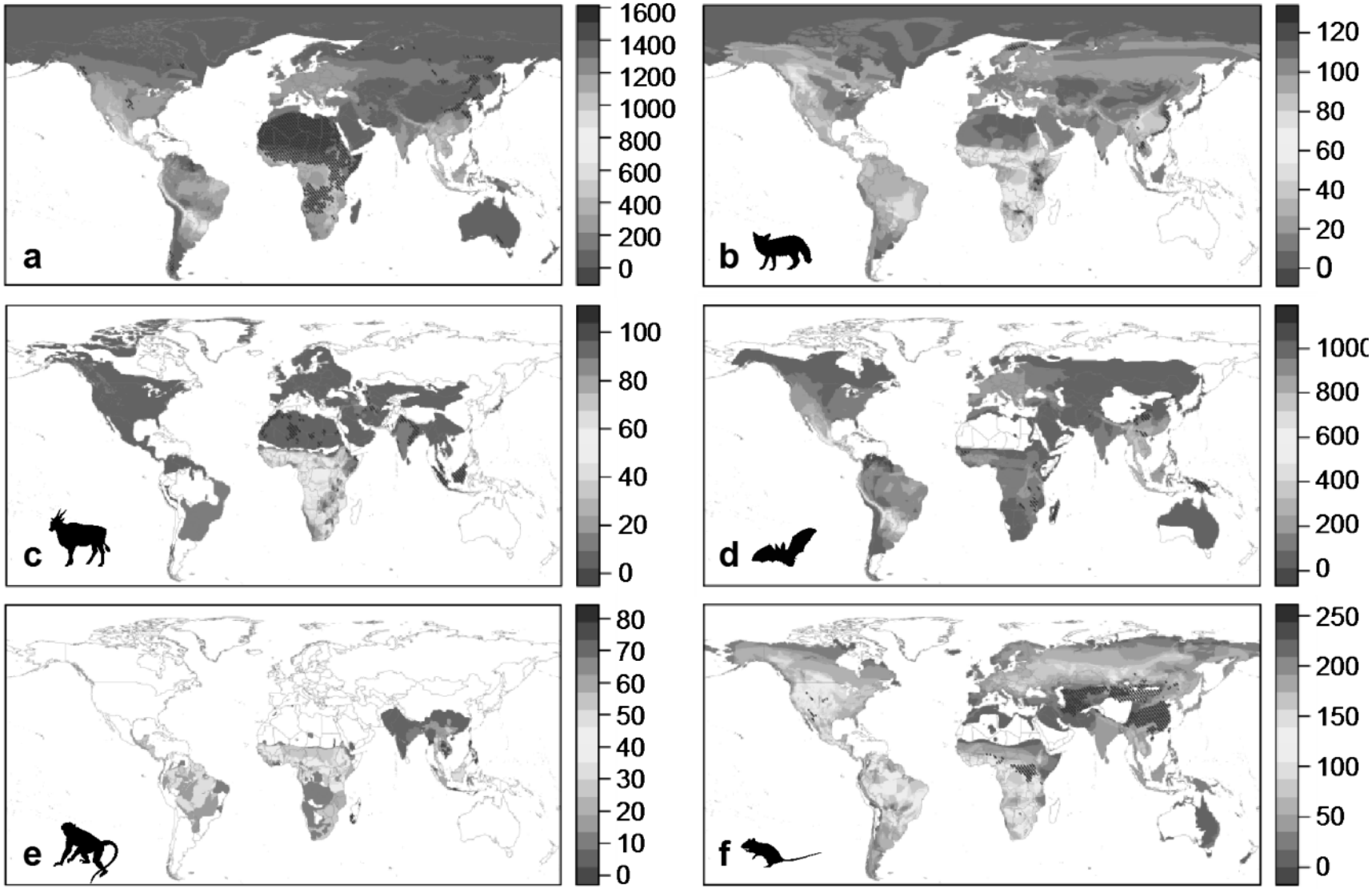
Olival et al. 2017, *Nature*

Predictors of proportion zoonotic per spp.



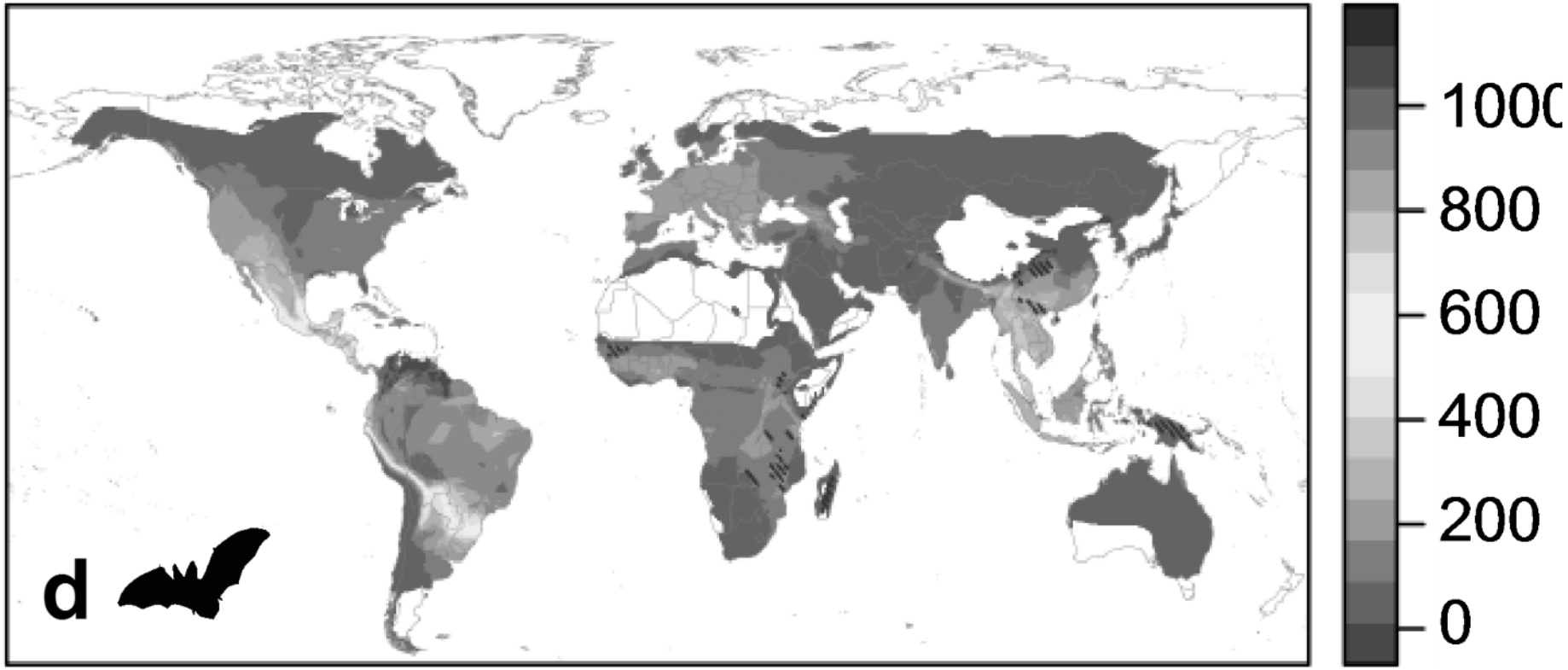
Olival et al. 2017, *Nature*

Missing Zoonoses - Mammals



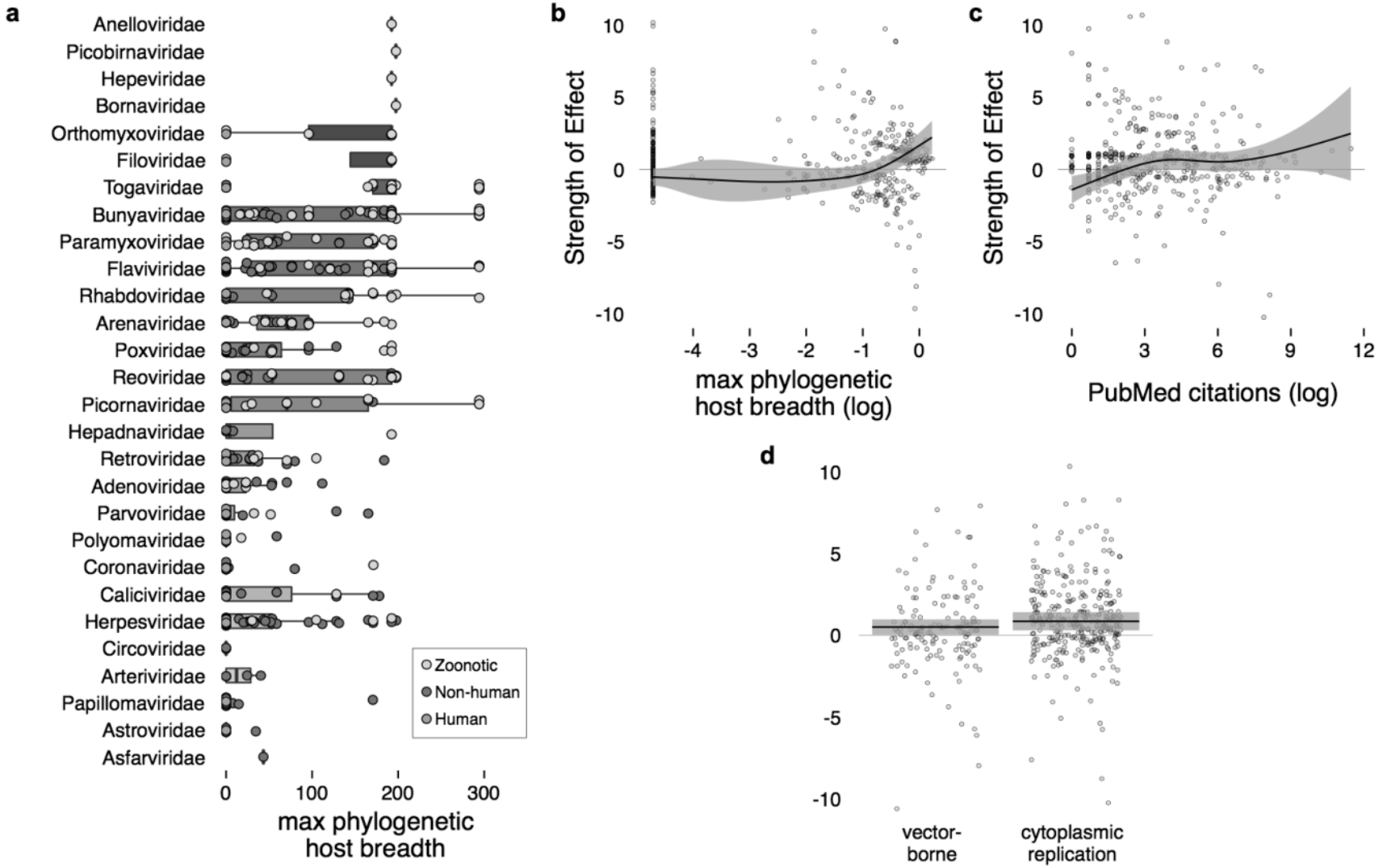
Olival et al. 2017, *Nature*

Missing Zoonoses - Bats



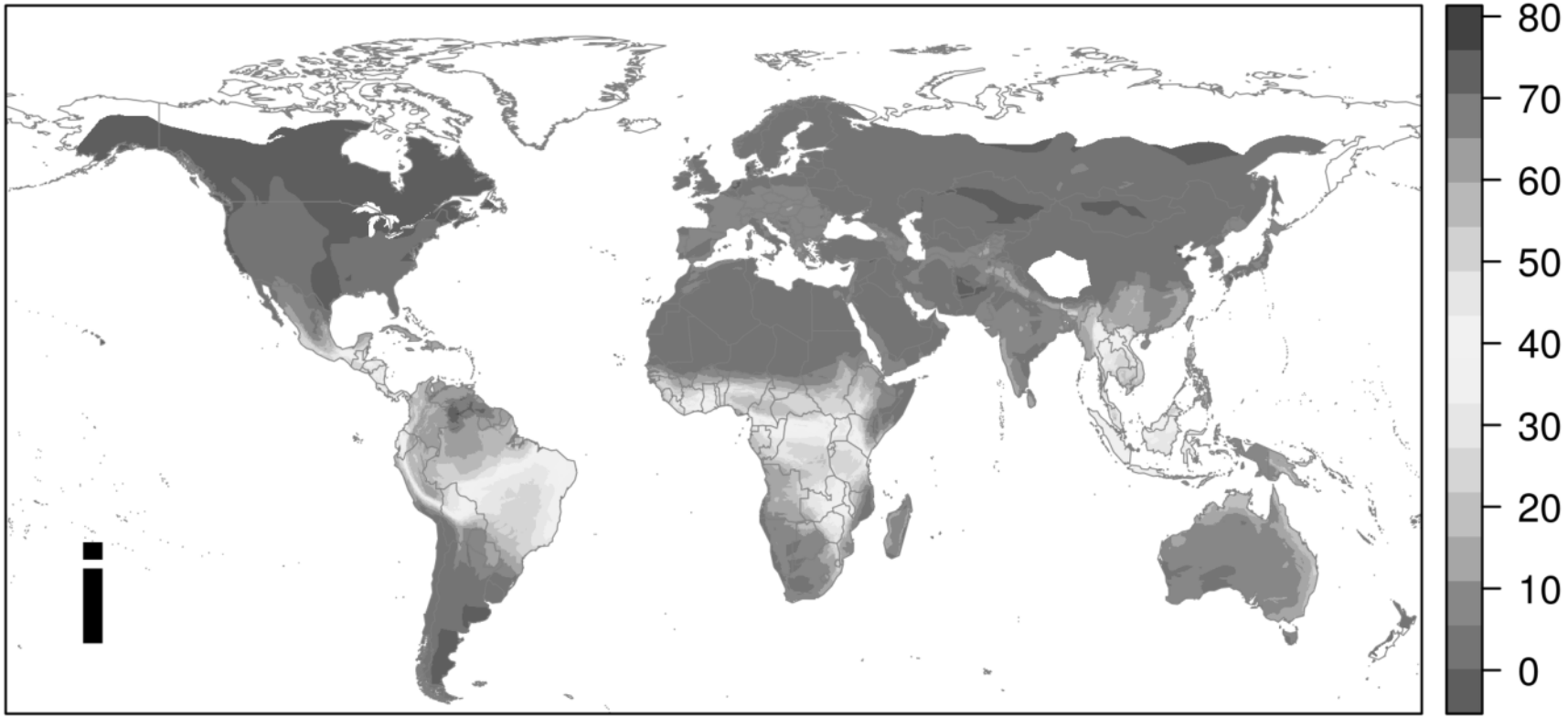
Olival et al. 2017, *Nature*

Viral Traits – Zoonotic potential



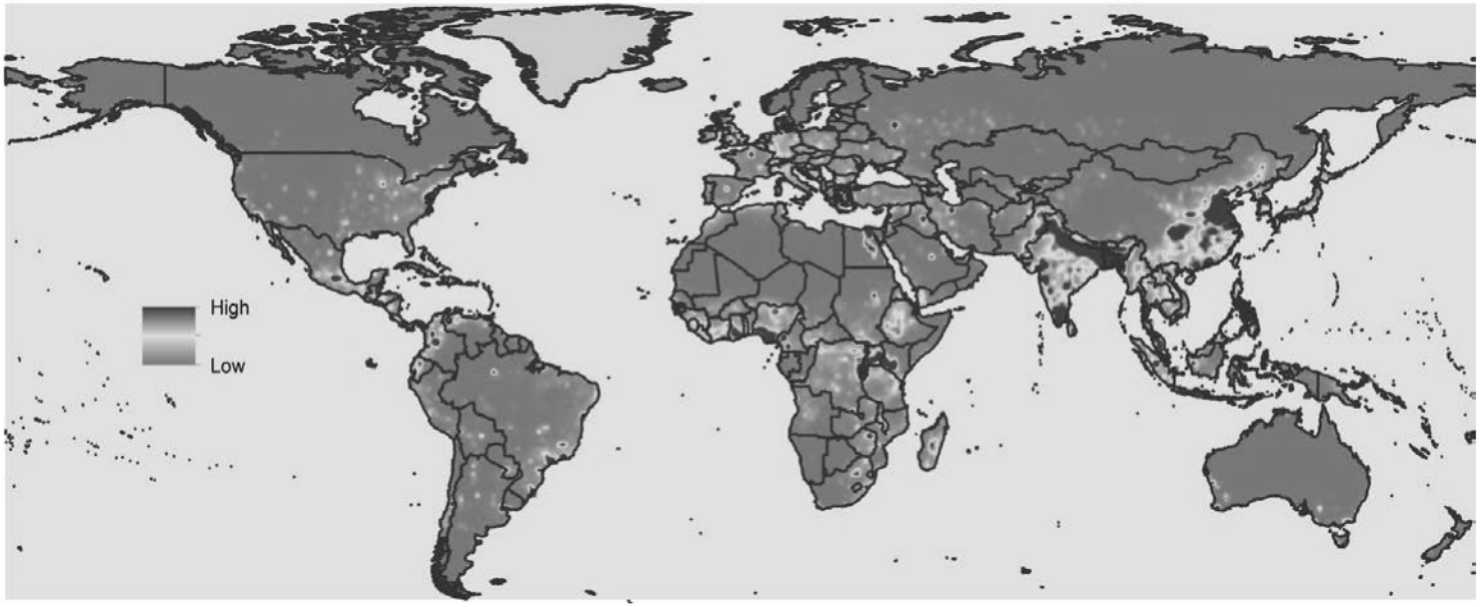
Olival et al. 2017, *Nature*

Gaps: Bat spp. unstudied for viruses

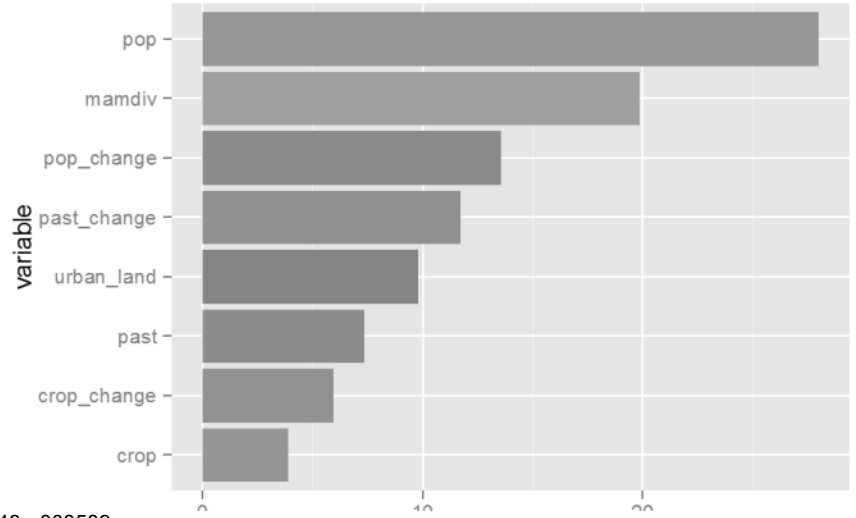


Olival et al. 2017, *Nature*

Emerging disease hotspots v.2.0



	relative influence (%)	std. dev.
population	27.99	2.99
mammal diversity	19.84	3.30
change: pop	13.54	1.54
change: pasture	11.71	1.30
urban extent	9.77	1.62



NIH 57943 - 003589

Allen *et al.* Nature Comm. *In press*

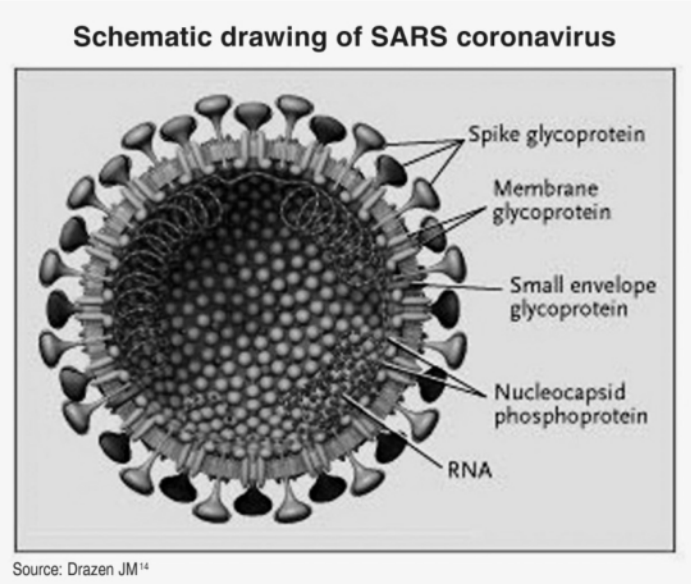
Severe acute respiratory syndrome (SARS)

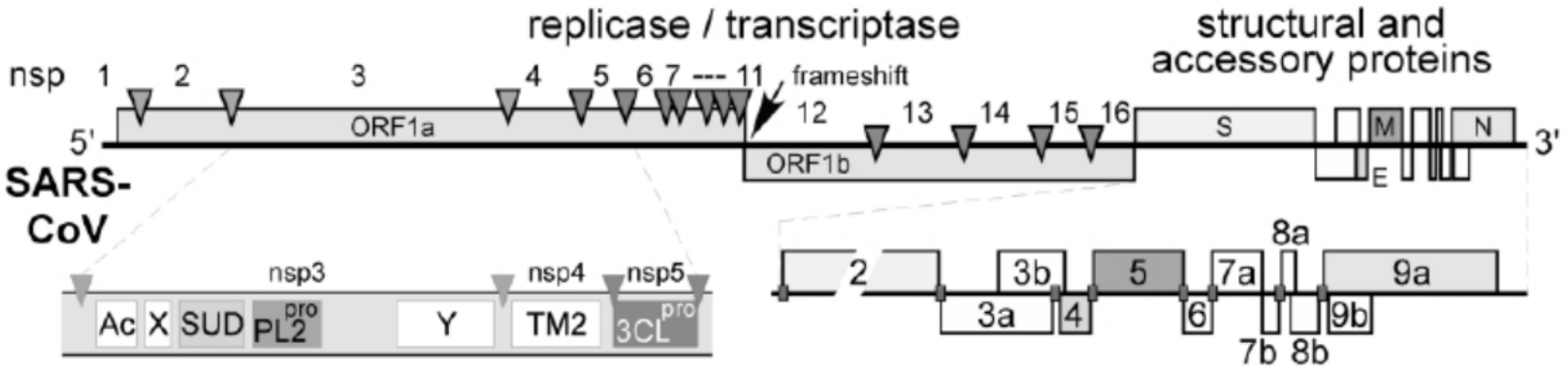
32 countries and regions involved

8096 cases globally, 774 deaths

7429 cases in China, 685 deaths

SARS coronavirus (SARS-CoV)





Snijder et al., JMB, 2003

Prevalence of diverse SL-CoVs in horseshoe bats, China

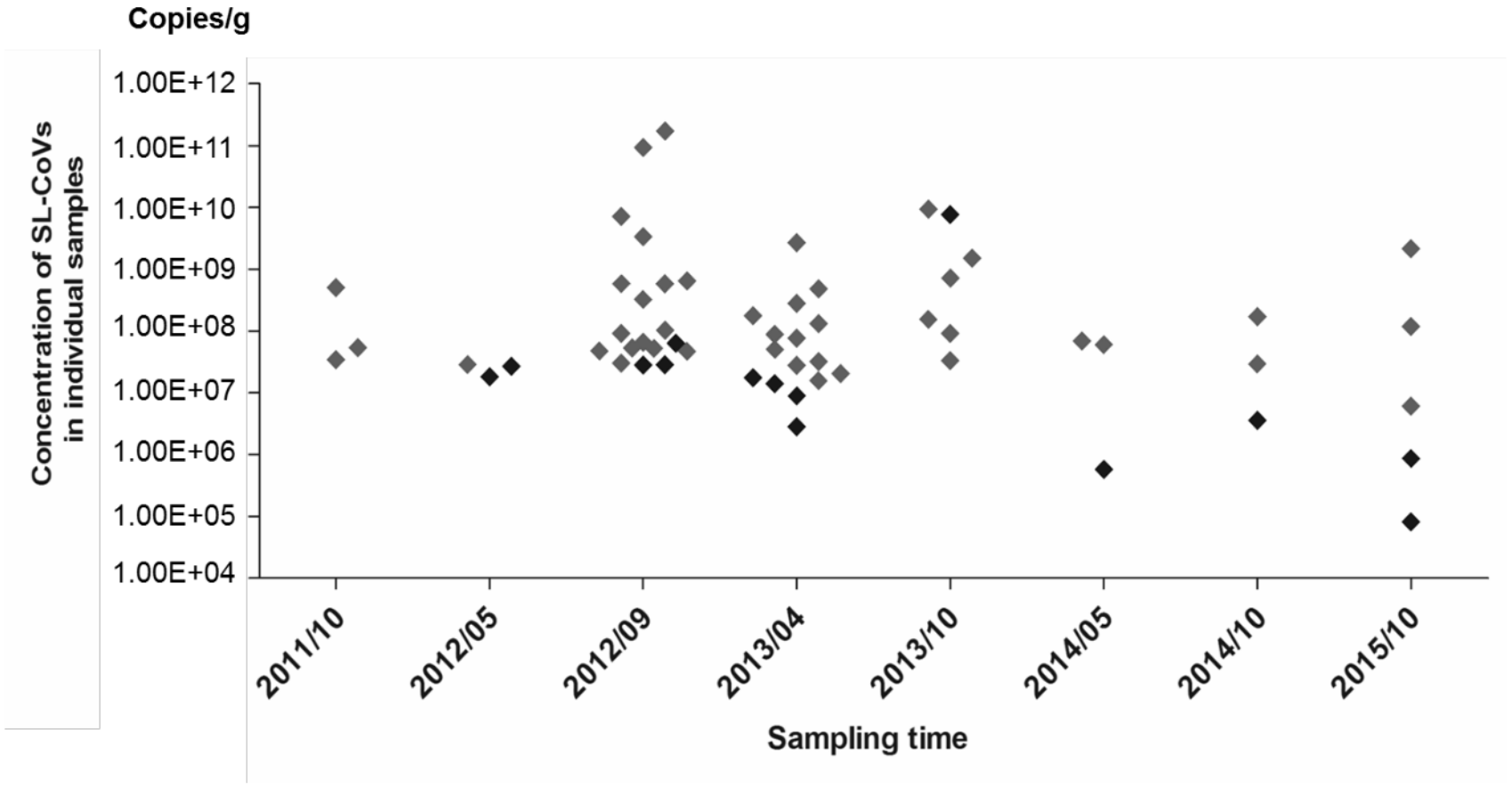
SARS-like CoV	Host origin	Location
Rf_273_04(Rf1)	*R. ferrumequinum*	Hubei
Rm_279_04(Rm1)	*R. macrotis*	Hubei
Rs_457_04(Rp3)	*R. sinicus*	Guangxi
Rs_HKU3-1_04	*R. sinicus*	Hong Kong
Rs_672_06	*R. sinicus*	Guizhou
Rs_806_06	*R. sinicus*	Hubei
Rs_3367	*R. sinicus*	Yunnan
Rs_SHC014	*R. sinicus*	Yunnan

Li et al., Science, 2005; Lau, et al., PNAS; Ren et al., J Gen Virol. 2006; Yuan et al., J Gen Virol. 2010; Ge *et al*., Nature, 2013

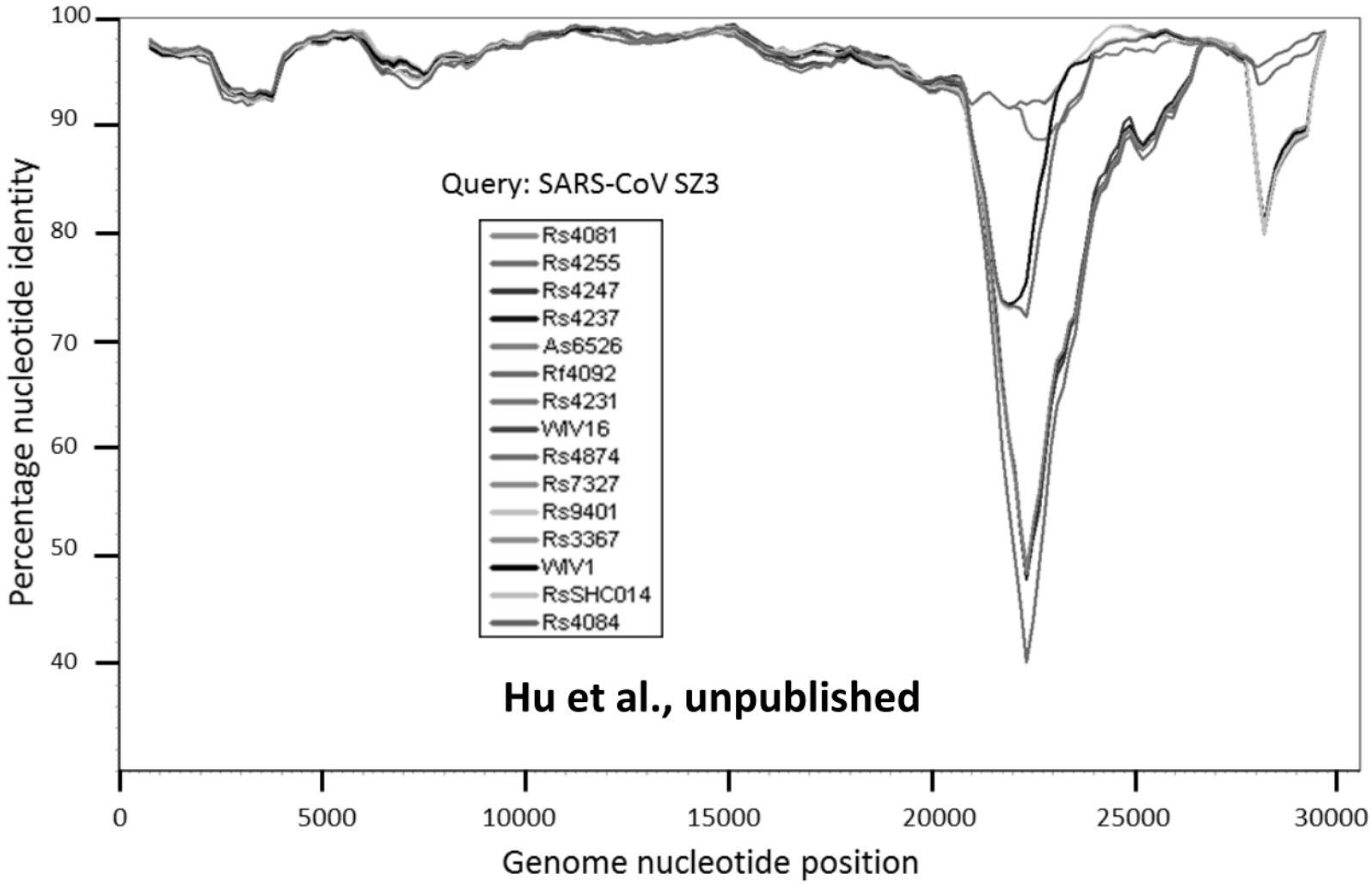
Longitudinal study of SL-CoVs in a bat cave

Sampling time	Sample amount	No. SL-CoV positive （%）	Bat species of SL-CoV positive samples
April, 2011	14	1(7.1)	*R.sinicus*
October, 2011	8	3(38)	
May, 2012	54	4(7.4)	
September, 2012	39	19(48.7)	*R.sinicus* *R.ferrumequinum*
April, 2013	52	16(30.8)	*R.sinicus*
July, 2013	115	8(7.0)	
May, 2014	131	4(3.1)	*A.stoliczkamus* *R.affinis*
October, 2014	19	4(21.5)	*R.sinicus*
May, 2015	145	0（0）	*Hipposideros.spp*
October, 2015	25	5（20）	*R.sinicus*
Total	602	64	

Quantification of SL-CoVs in the longitudinal study

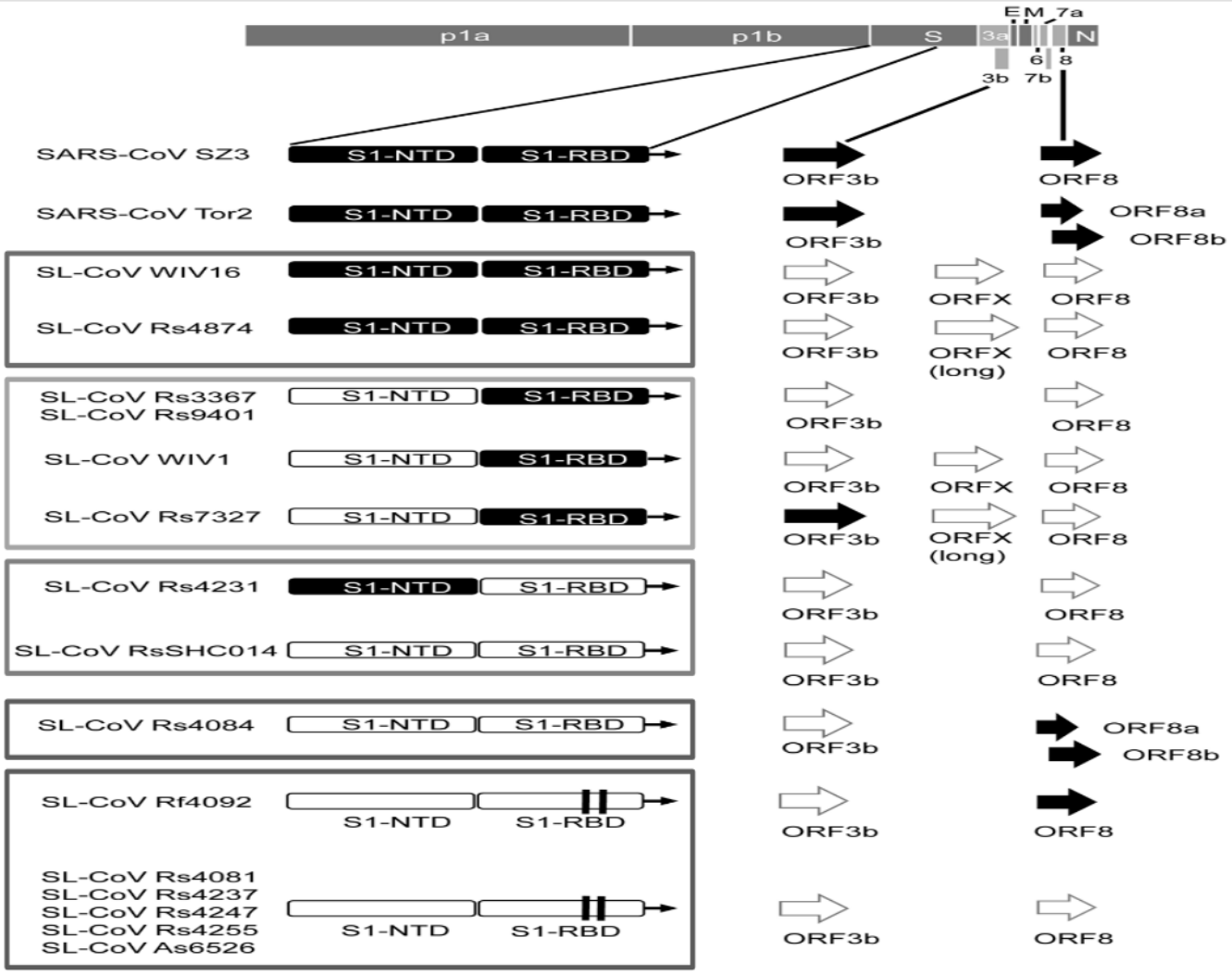


Genetically diverse SL-CoVs in a cave



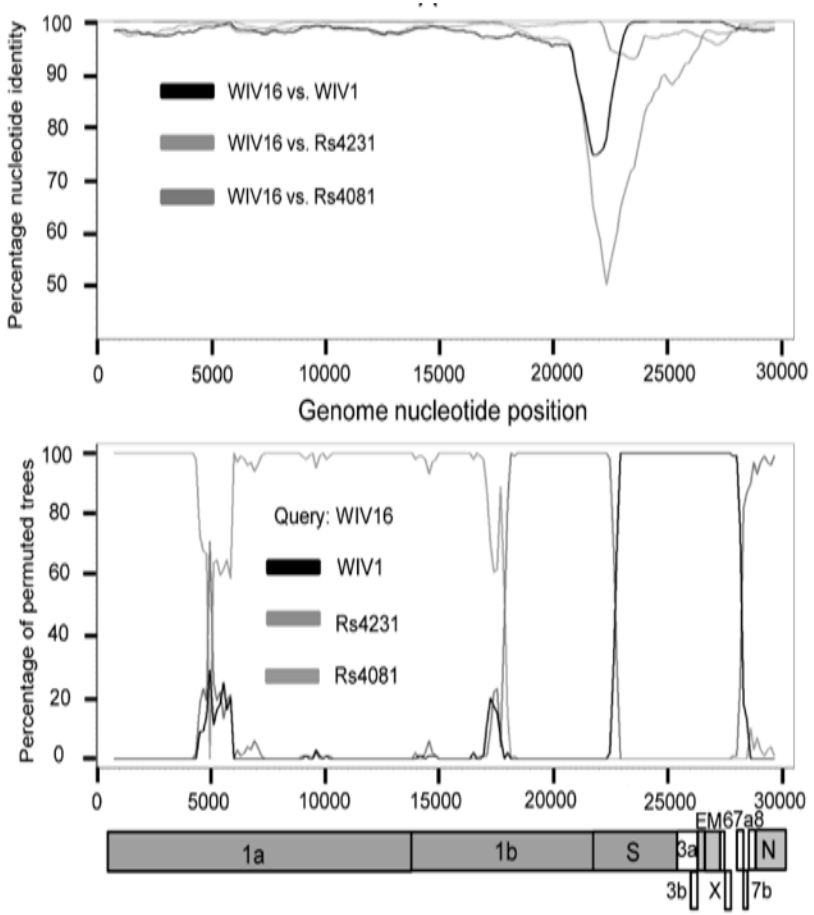
Hu et al. Unpublished data

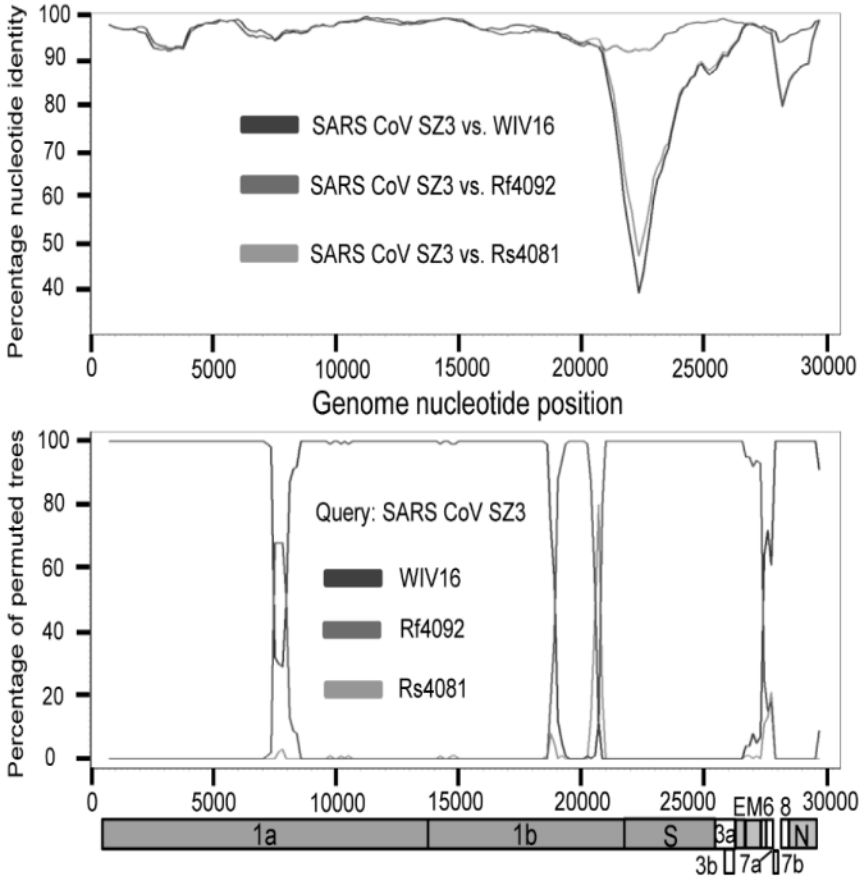
Genetically diverse SL-CoVs in a cave



Hu et al. Unpublished data

SARS-CoV derived from multiple recombination of SL-CoVs



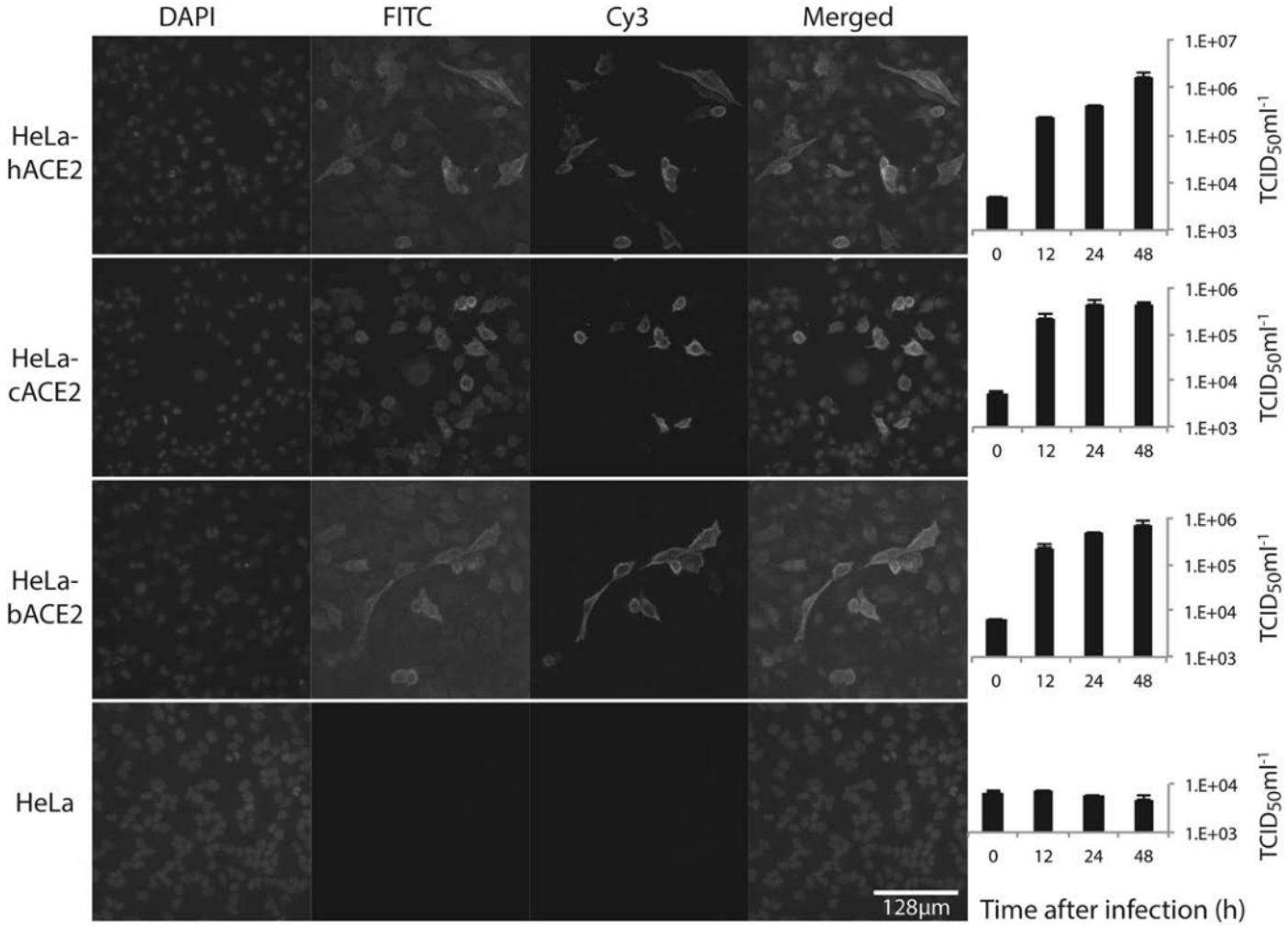


WIV16: WIV1 and Rs4231

SARS-CoV SZ3: WIV16 and Rf4092

Hu et al. Unpublished data

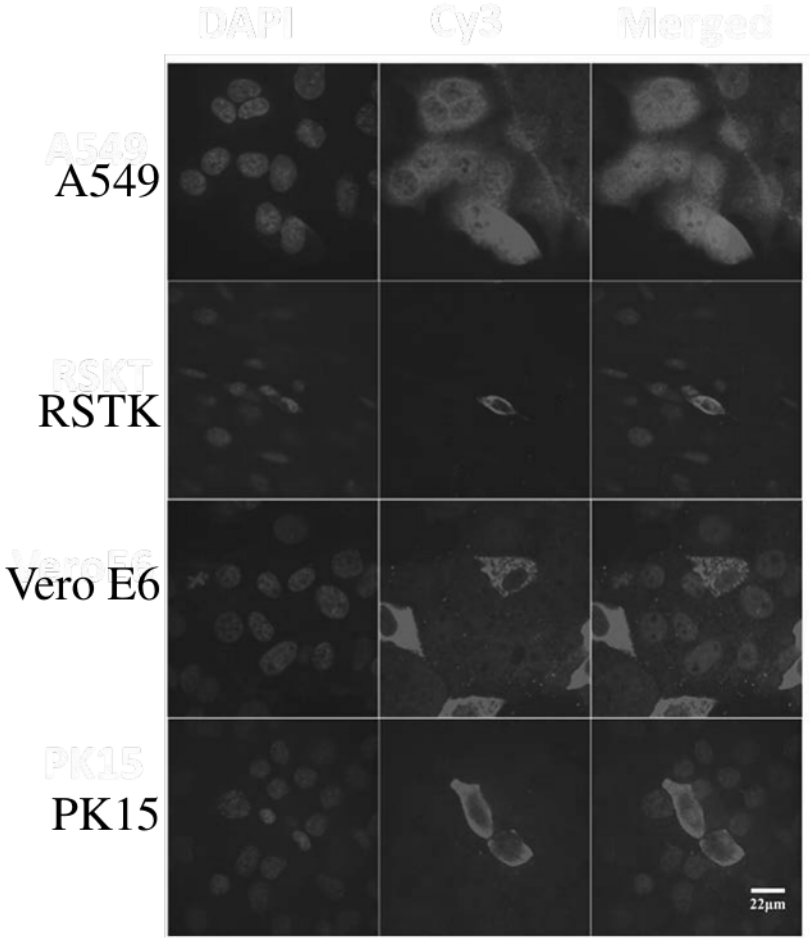
2 isolates SL-CoV WIV1 &16 use same receptor, ACE2, as SARS-CoV



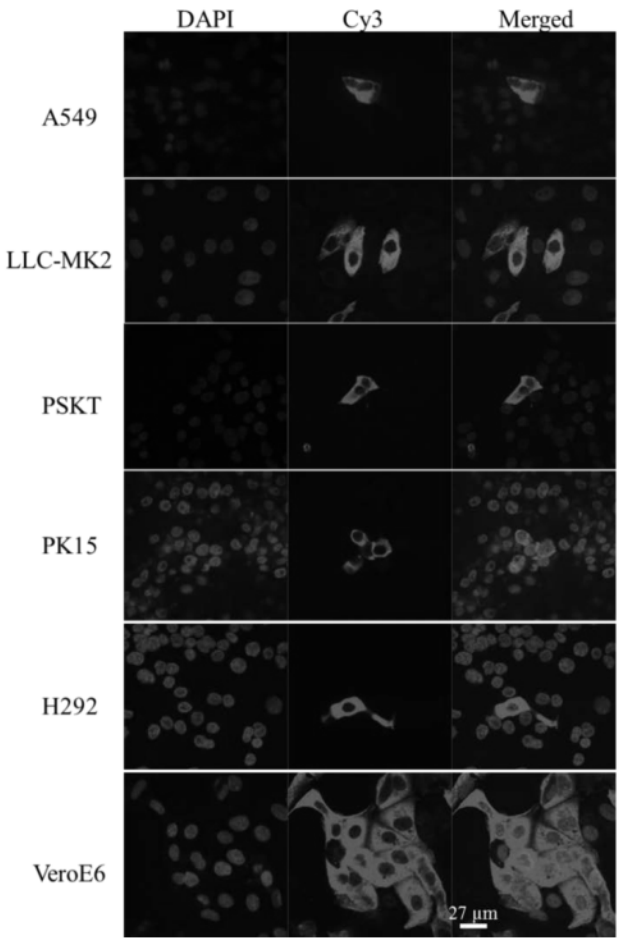
NIH 57943 - 003598

SL-CoV WIV1 &16 have wide host ranges

BtSL-CoV WIV1

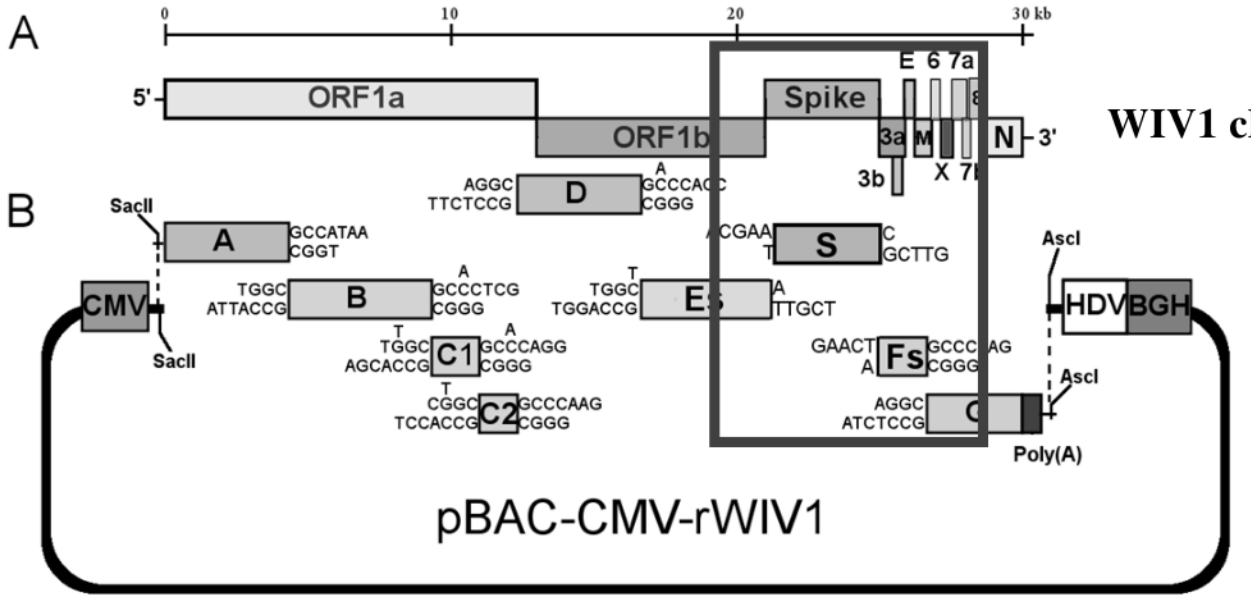


BtSL-CoV WIV16



Ge *et al.*, Nature, 2013; Yang, *et al.*, JVI, 2016

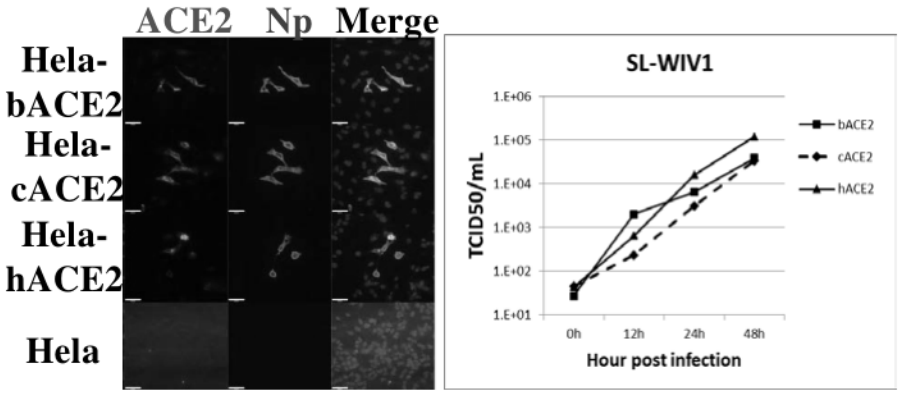
Construction of recombinant SL-CoVs by reverse genetic technique

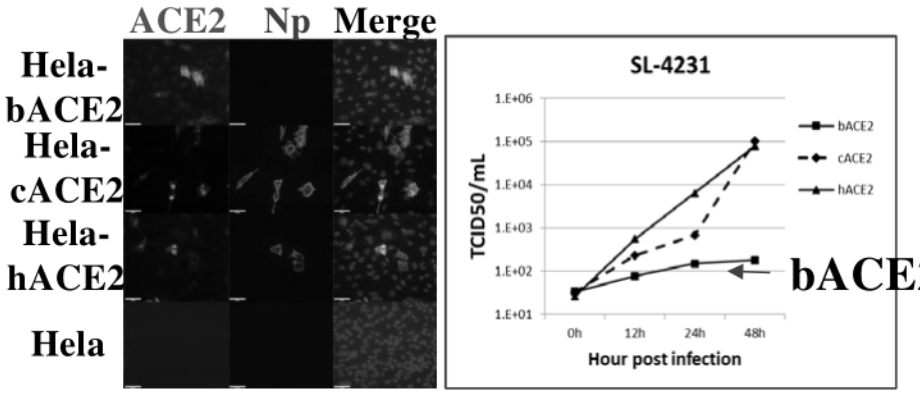


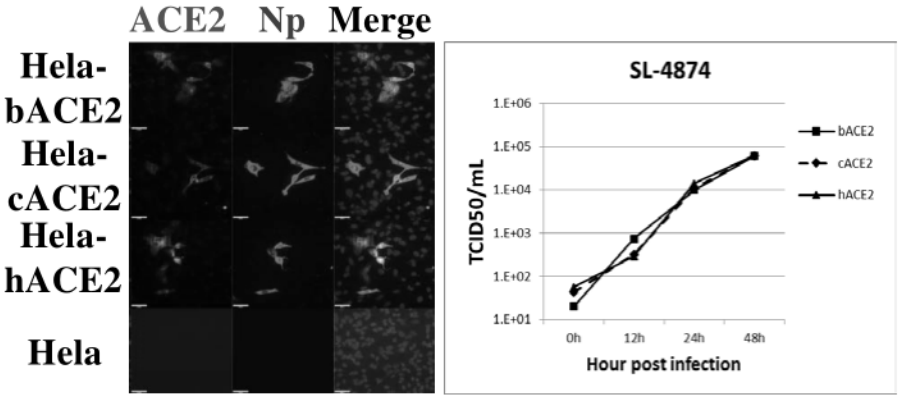
WIV1 cDNA clone

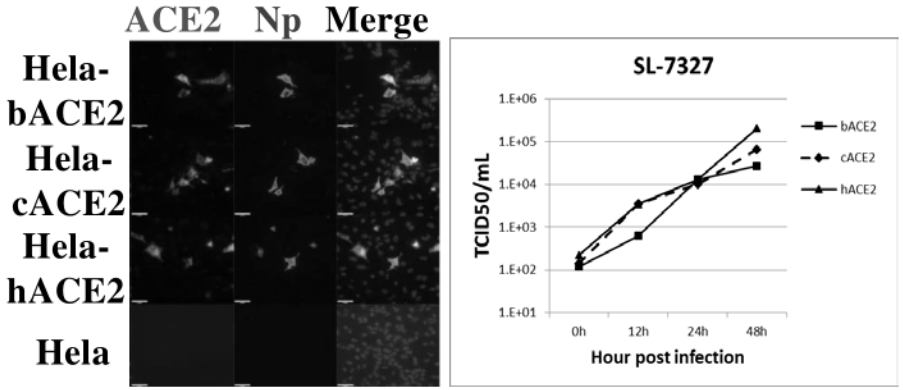
GD02	NTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYR	
WIV1	........Q.........S.............................F............I.N.....	**Y**
Rs4874	........Q.........S.............................F............I.N.....	**Y**
Rs7327	..................S.............................F..............N.....	**Y**
Rs4231	..NSK.S........L.....RS..N........DI...G.QS..AIGP...N..RP......A...H....	**Y**
Rp3	..AKQ.QGQ-----.Y..SH.KT.....----------SDE-.GVRT.ST.D..P.VP.A..AT.	**X**

ACE2 usage of recombinant SL-CoVs





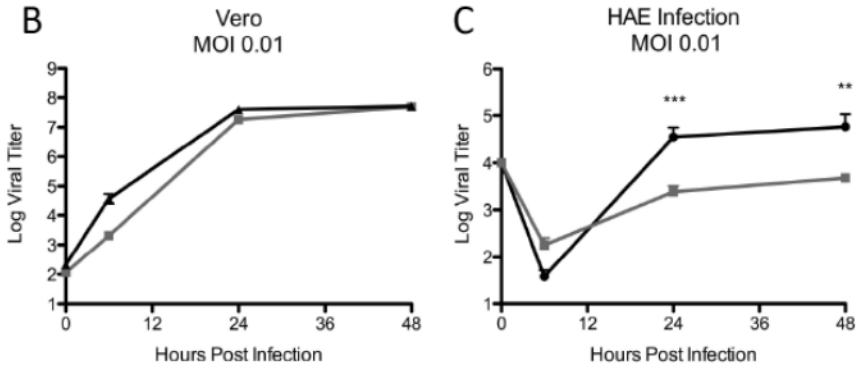


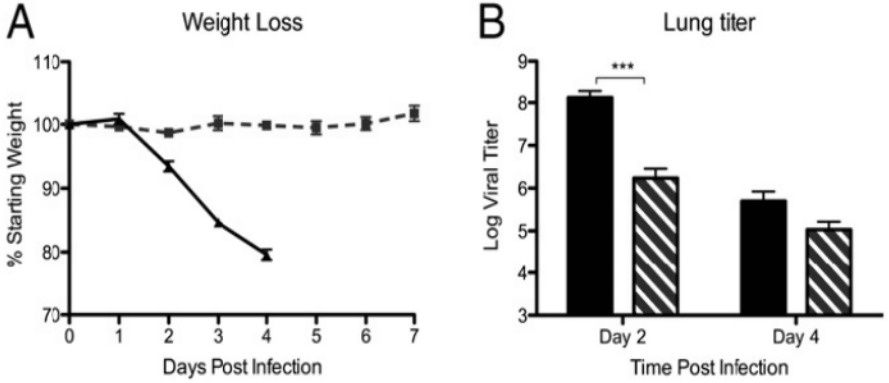


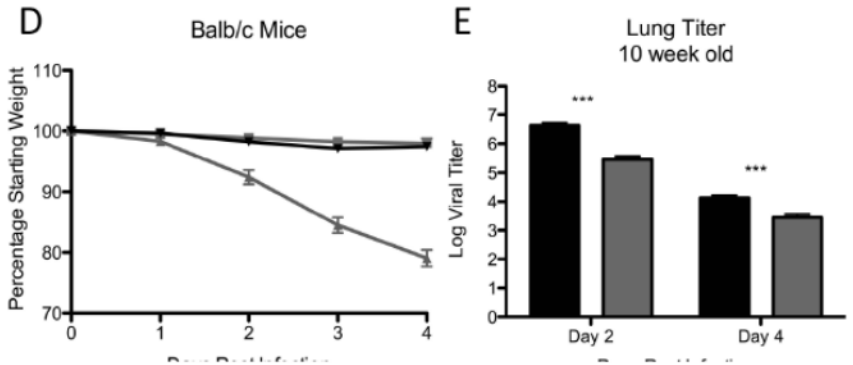
MOI = 1.0

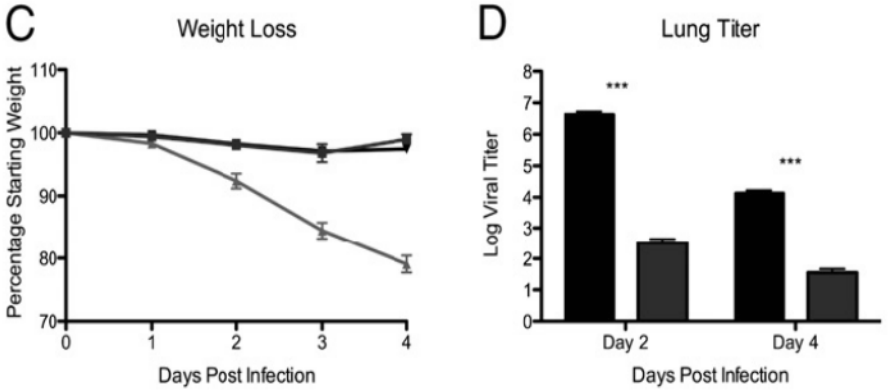
Hu et al. Unpublished data

Pathogenesis of SL-CoVs in transgenic mice









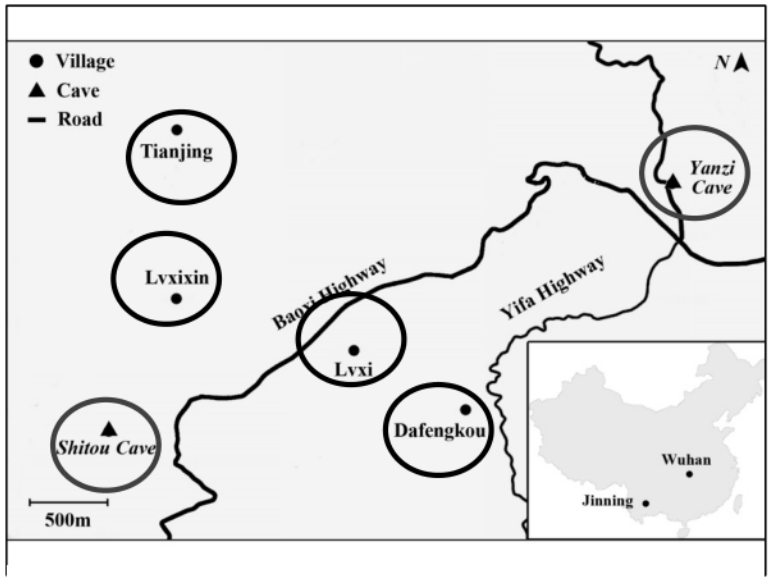
SARS-CoV and SHC014

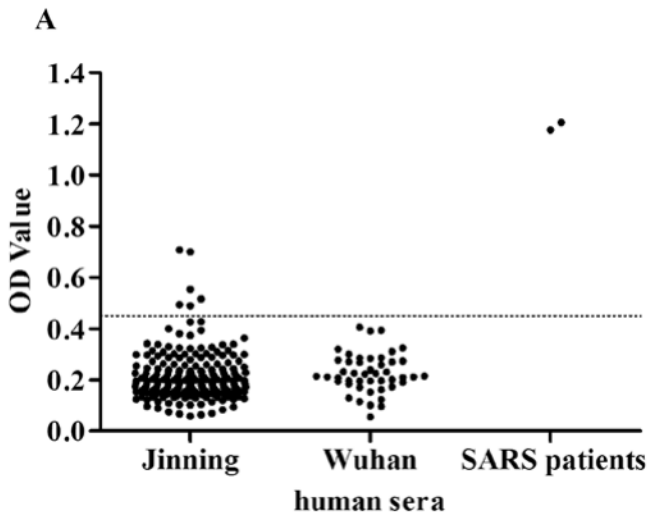
SARS-CoV and WIV1

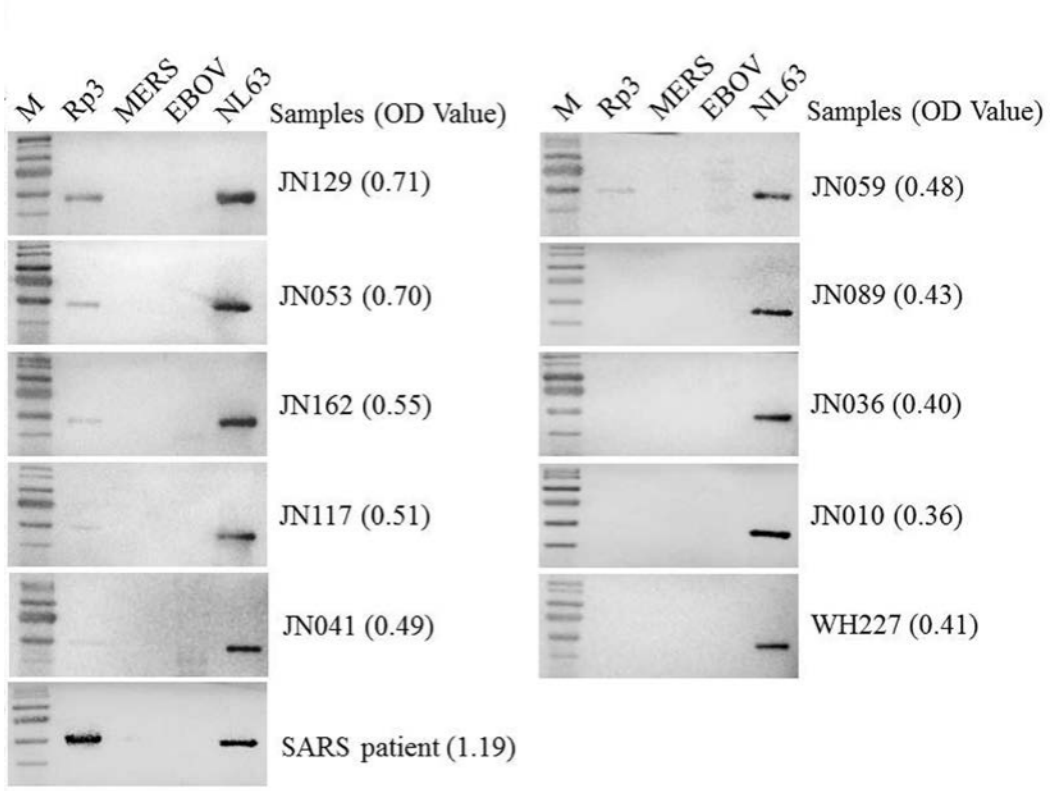
With the collaboration of Prof. Ralph Baric in North Carolina University

Menachery *et al.*, Nat Med, 2015; PNAS, 2016

Serological evidence of SL-CoV infection in human

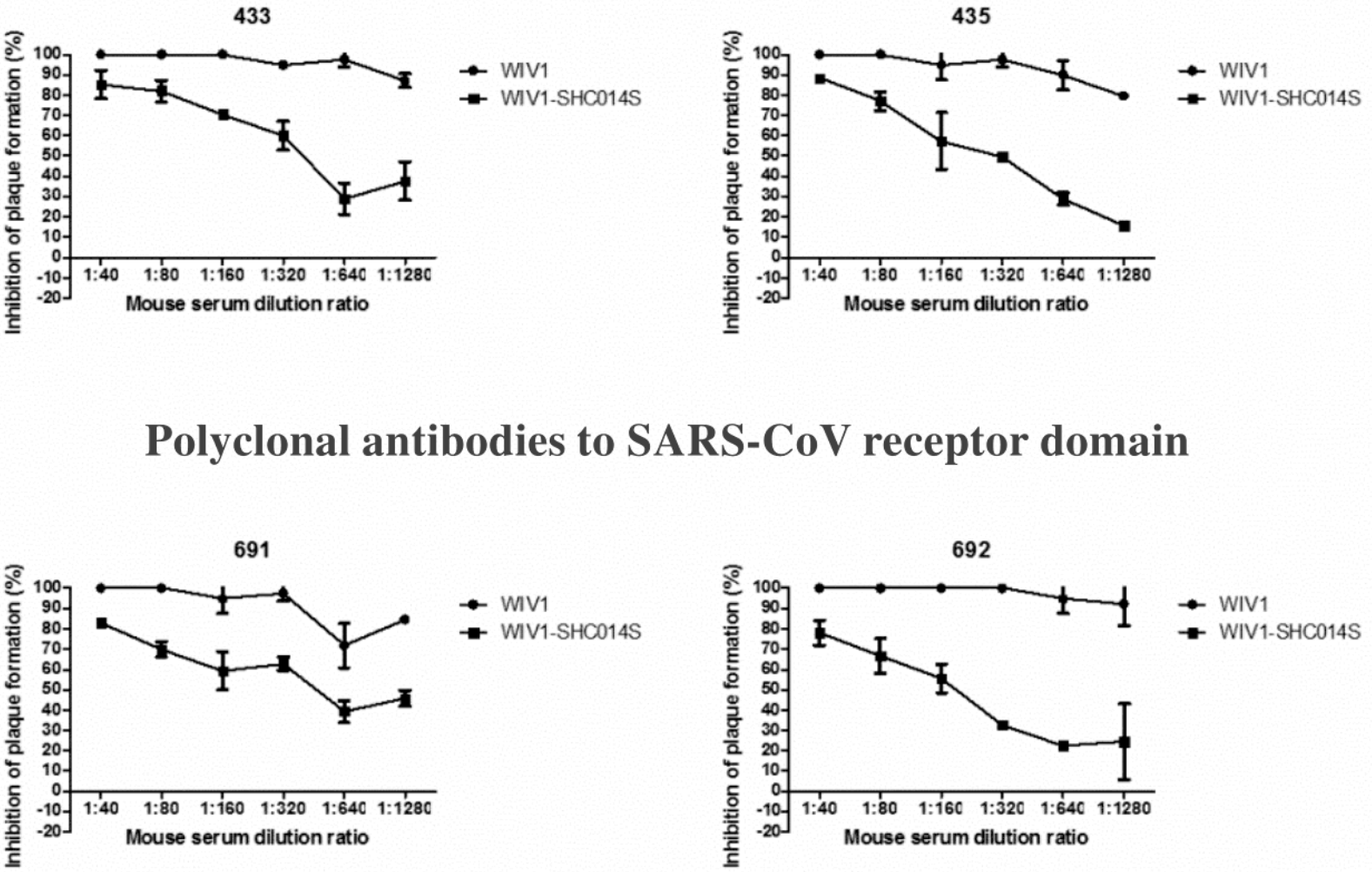






NIH 57943 - 003603

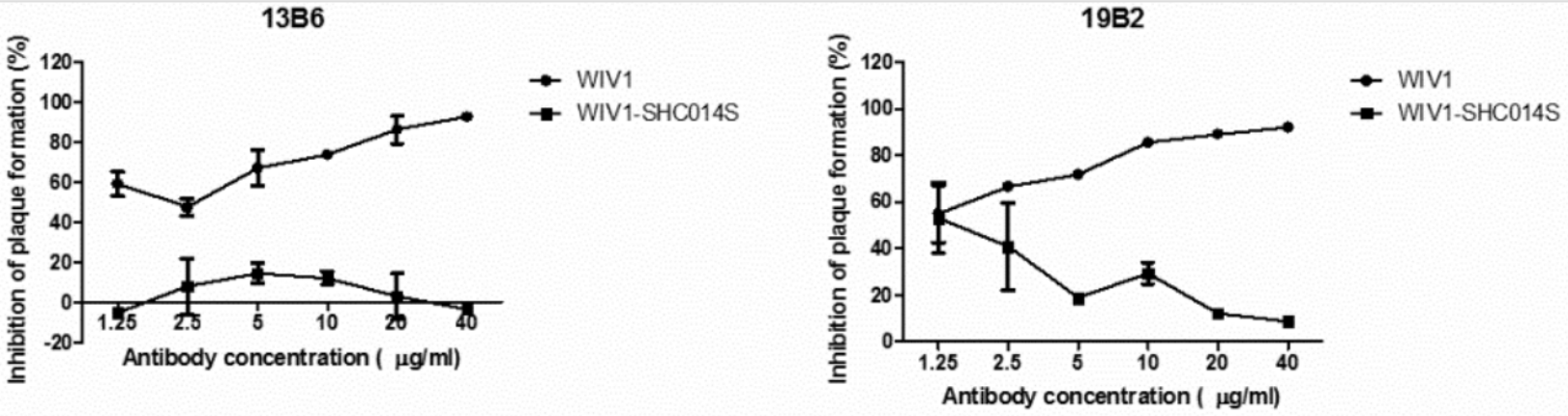
Cross-neutrolization assay of SASR-CoV antibodies against SL-CoVs



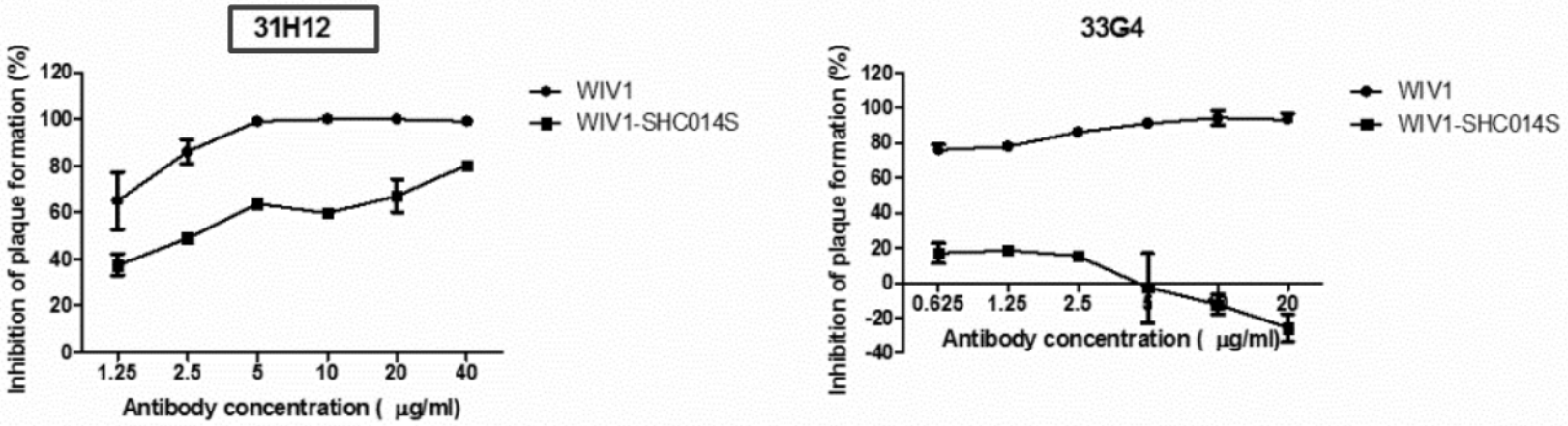
Polyclonal antibodies to SARS-CoV receptor domain

With the collaboration of Prof. Lanying Du and Shibo Jiang
Zeng et al., Unpublished results

Cross-neutrolization assay of SASR-CoV antibodies against SL-CoVs

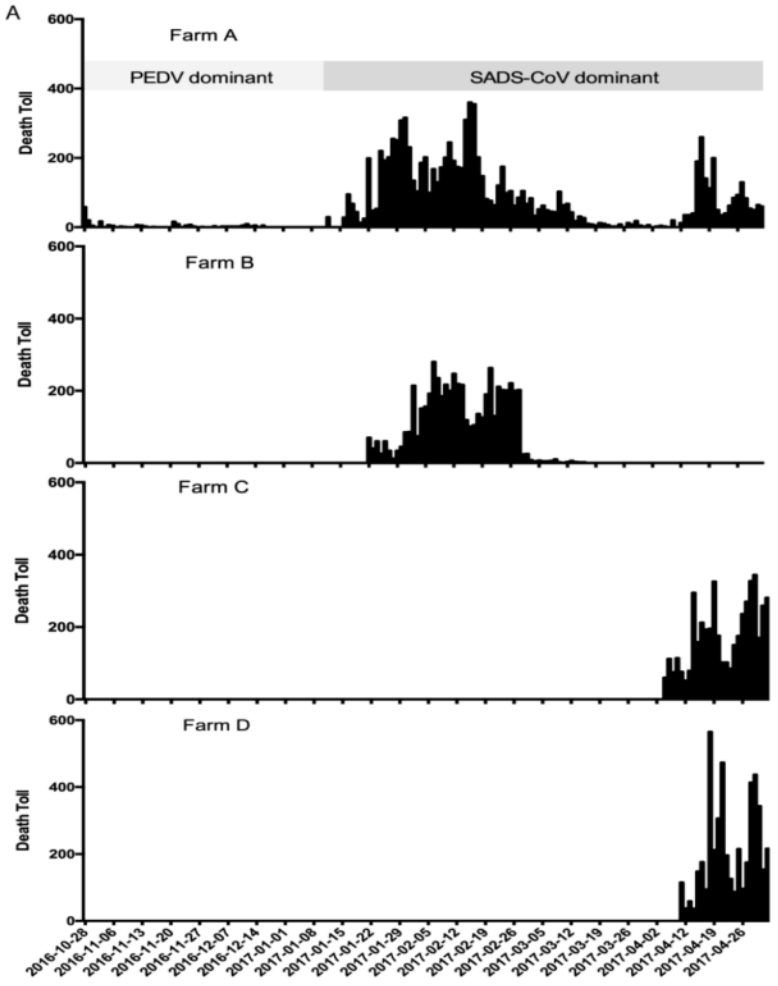


Monoclonal antibodies to SARS-CoV receptor domain



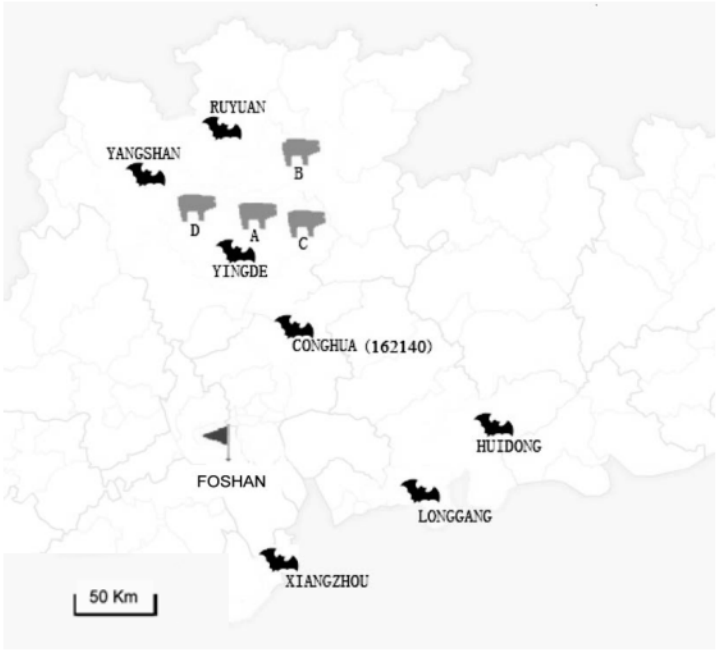
With the collaboration of Lanying Du and Shibo Jiang,
Zeng et al., Unpublished results

Severe acute diarrhea syndrome (SADS)



- From 28 October 2016, fatal swine disease outbreaks were observed in a pig farm in Qingyuan, Guangdong Province, China
- On 2nd May 2017, the disease has resulted in the death of 24,693 piglets from four farms. In Farm A alone, 64% (4659/7268) of all piglets born in February died.
- A coronavirus similar to bat CoV HKU2 was detected in diseased pigs

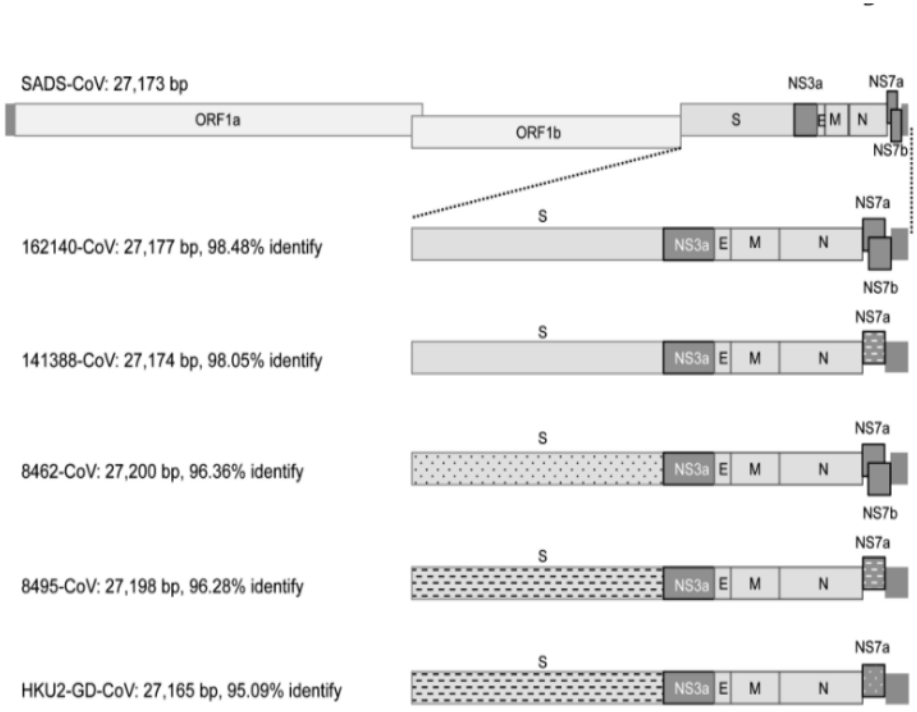
Detection of SADS-CoV in bats



Sampling			PCR analysis	
Time (Year)	Location	Bat Species	Fecal swabs sampled	PCR Positive
2013	Yingde	Rhinolophus sinicus	1	1
2013	Yangshan	Hipposideros pratti	36	1
2013-2016	Ruyuan	Rhinolophus sinicus	27	6
		Rhinolophus affinis	11	2
		Rhinolophus pusillus	41	6
		Rhinolophus rex	9	7
2014-2015	Conghua	Rhinolophus sinicus	70	2
		Rhinolophus affinis	34	7
		Rhinolophus pusillus	11	2
2013-2015	Huidong	Rhinolophus sinicus	37	2
		Rhinolophus affinis	59	29
		Rhinolophus macrotis	15	2
		Rhinolophus pusillus	1	0
		Hipposideros pomona	2	0
		Myotis ricketti	84	1
Apr 14; Jun 15	Longgang	Rhinolophus sinicus	55	1
		Pipistrellus abramus	5	1
Sep 14	Xiangzhou	Hipposideros pomona	38	1
		Total	596	71 (11.9%)

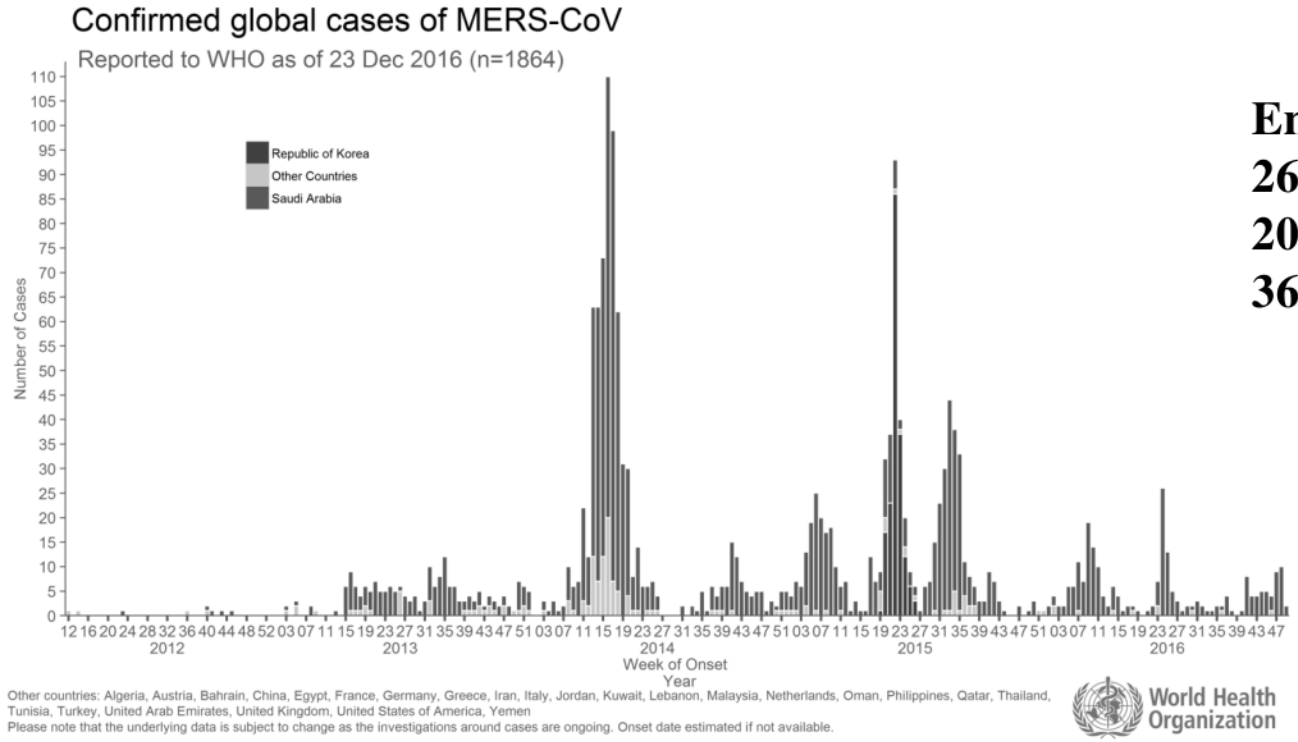
Map of Guandong Province

Diverse of SADS-CoV related viruses were detected in bats



Zhou et al., unpublished results

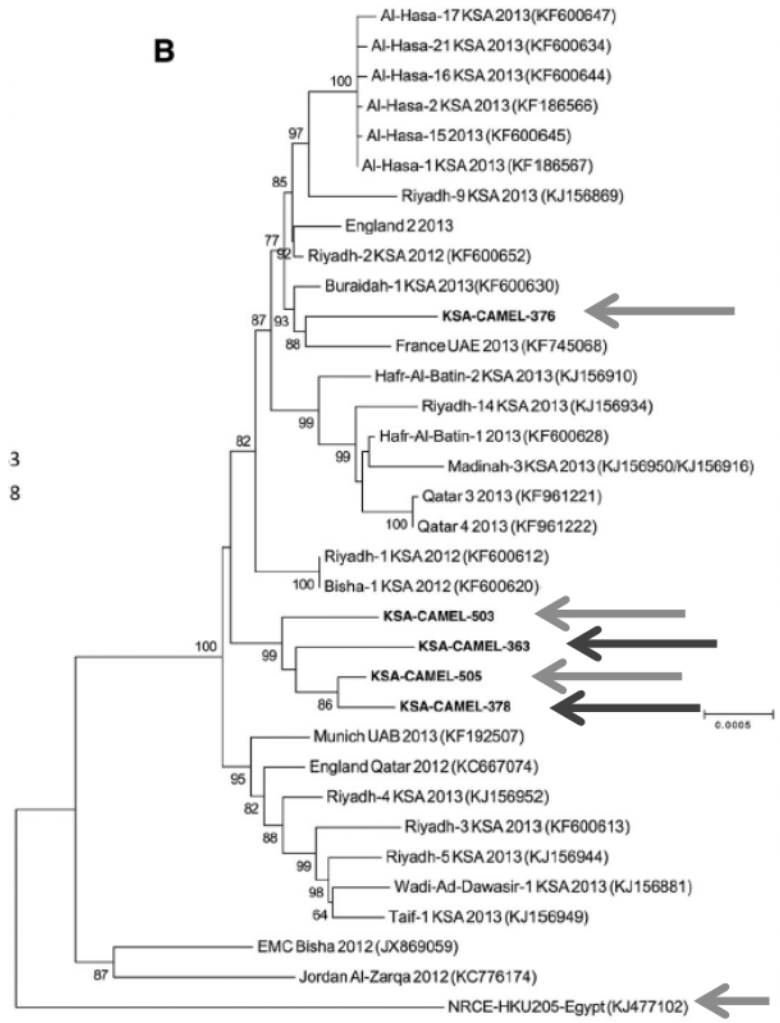
MERS and MERS-CoV



Emerged in Arabian Peninsula,
26 countries involved
2027 reported cases, 704 death
36% mortality

Zaki, et al New Eng J Med., 2012;
WHO (2017). Middle East respiratory syndrome coronavirus (MERS-CoV) – update, June-2017

MERS-CoV的传播



MERS Coronavirus Neutralizing Antibodies in Camels, Eastern Africa, 1983–1997

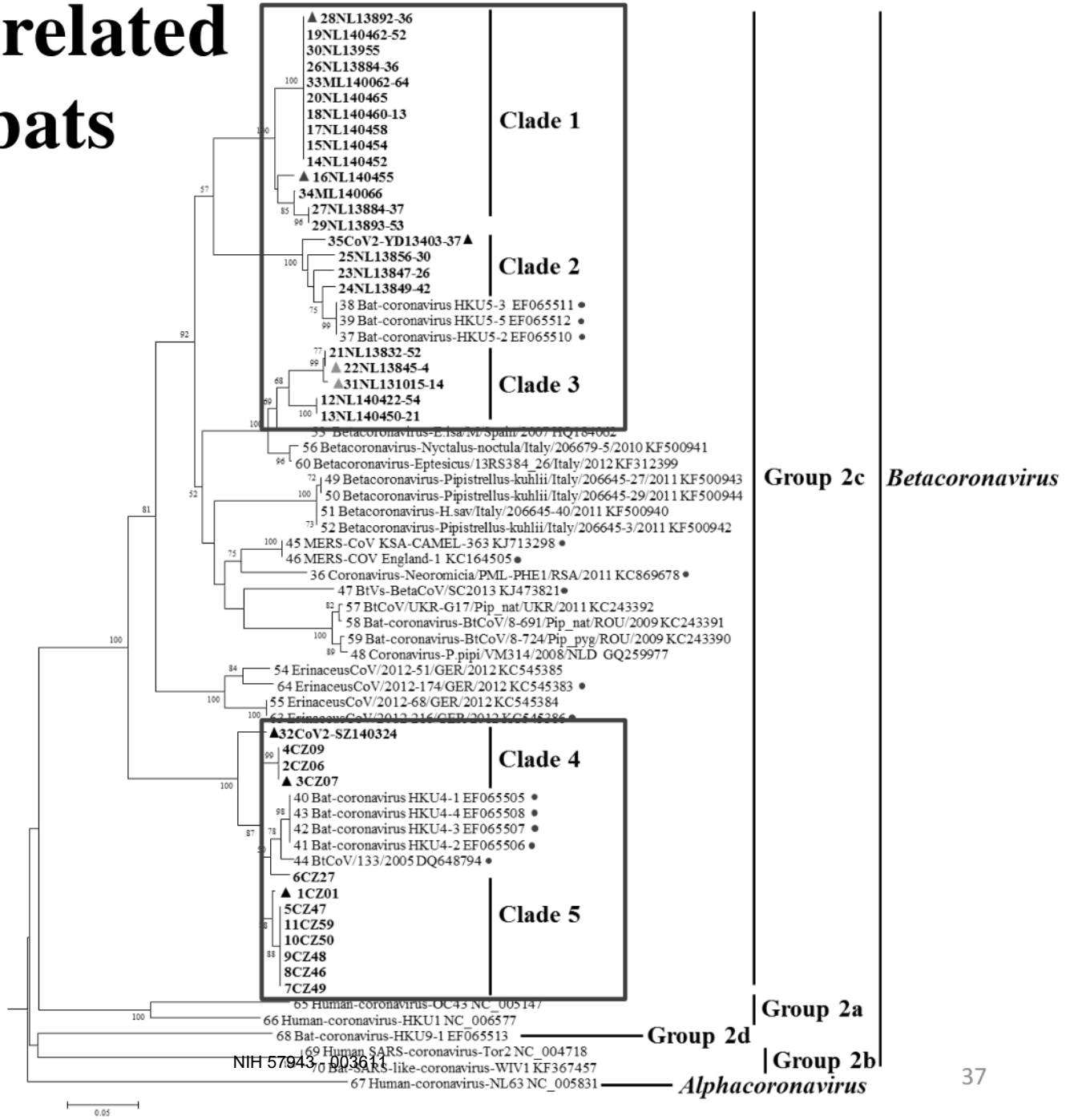
Marcel A. Müller,[1] Victor Max Corman,[1] Joerg Jores, Benjamin Meyer, Mario Younan, Anne Liljander, Berend-Jan Bosch, Erik Lattwein, Mosaad Hilali, Bakri E. Musa, Set Bornstein, and Christian Drosten

MERS Coronaviruses in Dromedary Camels, Egypt

Daniel K.W. Chu,[1] Leo L.M. Poon,[1] Mokhtar M. Gomaa, Mahmoud M. Shehata, Ranawaka A.P.M. Perera, Dina Abu Zeid, Amira S. El Rifay, Lewis Y. Siu, Yi Guan, Richard J. Webby, Mohamed A. Ali, Malik Peiris, and Ghazi Kayali

Azhar, et al., N Engl J Med. 2014; Briese et al., mBio 2014

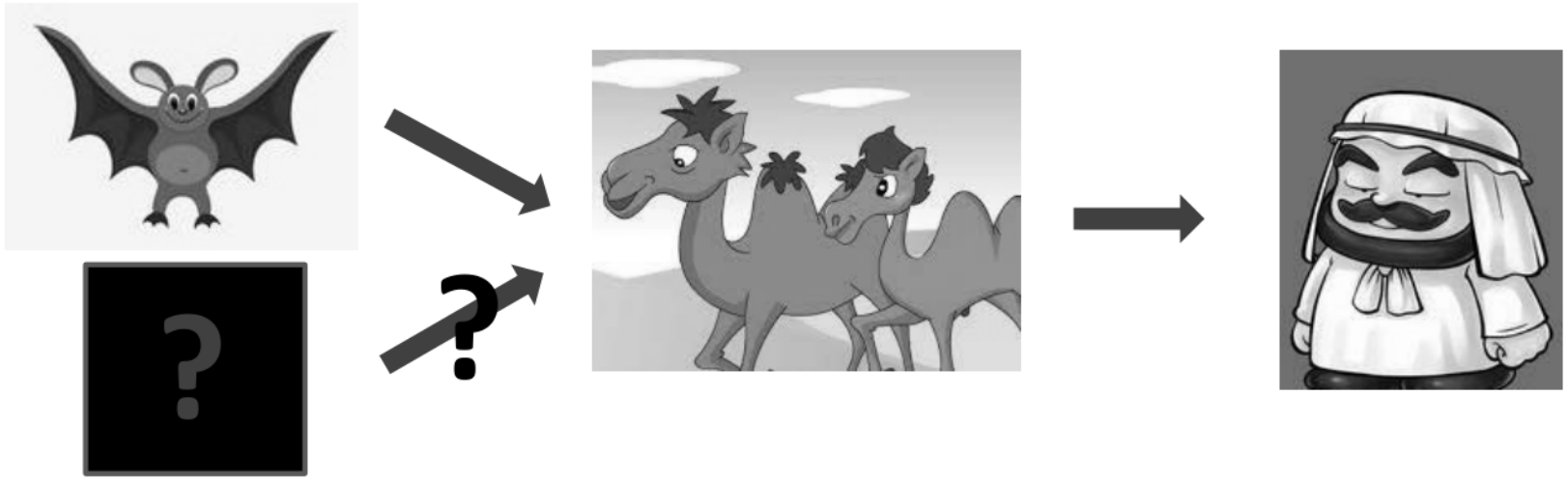
MERS-CoV related CoVs in bats



RdRp tree (440 bp)

Luo et al., unpublished result

MERS-CoV transmission



Summary

- **Bats are natural reservoirs of a diverse of coronaviruses**
- **Some coronaviruses have potential interspecies transmission to other animals and humans.**
- **Continued surveillance of bat coronaviruses, as well as the examination of human behavior risk for infection and serological survey are in need.**

Acknowledgements

- Prof. Lin-Fa Wang, Duke-NUS Medical School, Singapore
- Dr. Yunzhi Zhang, Yunnan Institute of Endemic Diseases Control and Prevention, Dali, Yunnan
- Dr. Peng Zhao, Wuhan Institute of Virology
- Dr. Kevin Olival, EcoHealth Alliance

Funding

- R01 AI079231 *Risk of viral emergence from bats* (Eun-Chung Park)
- R01 AI110964 *Understanding the risk of bat coronavirus emergence* (Erik Stemmy)

- NSF China
- National Basic Research, China
- USAID PREDICT
- DoD DTRA

NIH 57943 - 003614

From: Morens, David (NIH/NIAID) [E]
Sent: Fri, 6 Aug 2021 16:50:29 +0000
To: Wang Linfa; Stephen Goldstein; Jason Gale
Cc: (b)(6); Garry, Robert F; (b)(6); (b)(6)
Subject: RE: Chris Newman interview

Lin-Fa, not sure what this test is, but such a test, if it really correlates with Nt, it could be helpful in figuring out what caused the positive EIAs in the Cambodian populations our colleagues here have studied, these sera being strongly positive in spike and RBD EIA, but negative in Nt with an early SARS-CoV-2.

Stephen, you I think asked me a couple weeks ago whether they planned to publish this and I said I thought not, but now they have changed their minds, and are doing additional tests so they can publish. I will talk to the PI on a zoom call at 2 today.

David

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
☎ (b)(6) (assistants: Kimberly Barasch; Whitney Robinson)
301 496 4409
(b)(6)

From: Wang Linfa (b)(6)
Sent: Thursday, August 5, 2021 11:57 PM
To: Stephen Goldstein (b)(6); Jason Gale (b)(6)
Cc: (b)(6); Garry, Robert F (b)(6)
(b)(6) Morens, David (NIH/NIAID) [E] (b)(6);
Subject: RE: Chris Newman interview

Hi all,

We have developed a multiplex surrogate virus neutralization test platform which can detect specific neutralizing antibodies to different sarbecoviruses. The paper is coming out on 18 Aug and happy to discuss how we can use this novel approach to test different sera. The test is species independent and we have used it for human and more than 10 animal species.

Cheers,

LF

Linfa (Lin-Fa) WANG, PhD FTSE FAAM
Professor
Programme in Emerging Infectious Disease
Duke-NUS Medical School,
8 College Road, Singapore 169857
Tel: (b)(6)

From: Stephen Goldstein (b)(6)
Sent: Friday, 6 August 2021 10:13 AM
To: Jason Gale <j.gale@bloomberg.net>
Cc: (b)(6)
(b)(6) Wang Linfa
Subject: Re: Chris Newman interview

- External Email -

In terms of conclusions, I think if seropositivity among workers in the wildlife trade is higher than background it would be strongly suggestive of occupational, not just community, exposure.

Sent from my iPhone

On Aug 5, 2021, at 8:11 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I suspect that Xiao Xiao is connected to many of the vendors he was visiting and hanging out with via WeChat, so he would've been critical for contact-tracing and reaching these folks. I guess there's no upside for the vendors to voluntarily give blood to check for neutralizing antibodies etc. Plus is would be too long ago now to draw any conclusions, right?

From: (b)(6) At: 08/06/21 12:04:52 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: (b)(6)
(b)(6)
Subject: Re: Chris Newman interview

I'll let you know.

--

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T (b)(6)
E

On 6 Aug 2021, at 12:03 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Very interested to see that when it's ready, Eddie. The animals Xiao observed were clearly not in great shape, so no doubt stressed and shedding loads of whatever pathogens they were infected with. Plus, their fecal matter was dropping on animals stacked below them.

----- Original Message -----
From: Edward Holmes (b)(6)
To: (b)(6)
CC: JASON GALE, (b)(6),
(b)(6),
At: 08/06/21 12:00:45 UTC+10:00

Hard to interpret this. Could of course mean that the animals didn't have the virus BUT I'm involved in another project looking at market animals and I can tell you that these animals carry *a lot* of viruses. Not just coronas. Accident waiting to happen.

Cheers,

Eddie

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T (b)(6)
E

On 6 Aug 2021, at 11:57 am, Stephen Goldstein (b)(6) wrote:

Yes serosurveys back then showed >50% seroprevalence in traders specializing in civets. Clearly occupational exposure, and probably outside of just the SARS epidemic period. Serosurveys of everyone in the wildlife chain from farm to market are my number one dream study to crack this nut.

Sent from my iPhone

On Aug 5, 2021, at 7:50 PM, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

If I eventually become the 1000th journalist to write a book about SARS-CoV-2, this has got to make it in:

Chris Newman: *[00:29:10]* One thing he interestingly did tell us, and it was in our original paper but didn't make it into the scientific reports sort of sanitized version is that he (Xiao) knew these vendors very well. He would go and see them weekly. He was on first-name terms. They'd chat, have a cigarette and a drink together and so forth. None of them got sick. Not one of them got sick from coronavirus. So they were selling these animals, but they themselves didn't get it.

Am I right in thinking that a serosurvey of workers in the two wet markets in Guangdong implicated in the SARS outbreak found 30% had cross-reactive antibodies? Would be fasssssssscinating to know whether Wuhan's wildlife vendors had some level of immune protection from prior exposure to SARS-related coronaviruses.

JG

From: (b)(6) At: 08/06/21 09:57:55 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:) ,
(b)(6)
Cc: (b)(6) ,
(b)(6) ,
Subject: Re: Chris Newman interview

Agree with Eddie. They tests to do with those blood samples depending on quantity, storage, and availability would be to look for antibodies to SARS-CoV-2 rather than looking for evidence of the virus itself. But yes, I can imagine that being difficult or impossible in the current climate.

Stephen

From: Edward Holmes (b)(6)
Sent: Thursday, August 5, 2021 5:55:20 PM
To: Jason Gale
Cc: (b)(6) ; Peter Daszak; (b)(6) ; Wang Linfa; (b)(6) ; Stephen Goldstein
Subject: Re: Chris Newman interview

That's interesting Jason.

The blood samples could be very useful (depending on how they are stored) but they would to find a lab that is willing and able to look at them. Again, the politics could be tricky.

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T (b)(6)
E

On 6 Aug 2021, at 9:27 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Howdy,
I had a very interesting convo just now over Zoom with Chris Newman, the wildlife ecologist who worked on the Xiao paper in Scientific Reports. The publication's history is even more interesting than I thought. Couple of interesting things: the corresponding author Zhou was part of China's wildlife police/border control efforts (so knows a LOT!) and Xiao collected ticks from the wildlife he was surveying, so should have blood samples from infested animals from May 2017 until the market closure stopped data collection in Nov. 2019.
Jason

From: Morens, David (NIH/NIAID) [E]
Sent: Tue, 5 Nov 2019 15:05:15 -0500
To: Ellen Carlin
Bcc: Morens, David (NIH/NIAID) [E]
Subject: Re:

Ellen, i plan to be there so see you i hope

I emailed (b)(6) but daid i know you must be crazybusy so no need to reply now but plz send contact info when you are settled there

I have been promiding myself to go up to (b)(6) more often, like evry summer i hope. I also may heed to go up very soon to see (b)(6). You should go up some time and see (b)(6) and check the place out. If i am there I'll show you sone of the sights. Also i hope to go up to (b)(6) when (b)(6) is there and check thst out

A hydroplane race: that's the real thing to see! It defines the overused word AWESOME. d

David M Morens MD
OD, NIAID, NIH
Sent from my iPhone

> On Nov 5, 2019, at 14:34, Ellen Carlin (b)(6) wrote:
>

Ha, it's not boring, the stories are amazing! You should write them down as a sort of (b)(6). (Though the (b)(6) makes it sound more like New Orleans. Scary!!) I really enjoy maritime history and am going to read up on the Fitzgerald.

Thanks again for the compliments on the study and the paper. We'll see if we can get any purchase with it… A few of us on the paper will be at Cosmos tomorrow so we can talk more then about publishing prospects.

From: "Morens, David (NIH/NIAID) [E]" (b)(6)
Date: Friday, November 1, 2019 at 10:20 AM
To: Ellen Carlin (b)(6)
Subject: RE:

Ellen, yes, I plan to be there next week at the Cosmos Club.

The story of the Fitzgerald has become sort of a modern legend (see Wikipedia site: https://en.wikipedia.org/wiki/SS_Edmund_Fitzgerald)

(b)(6)

I have to say now, (b)(6) being up close to those moving freighters, whether in a small boat nearby or on the shore, is pretty awesome. When the big ships were loaded and speeding above the speed limit, which was almost always, (b)(6)

(b)(6)

(b)(6)

(b)(6)

Sorry if this is all boring, I am just (b)(6) I guess, and am still pumped about (b)(6) this summer. I am really fired to (b)(6)

Yes, the paper is really REALLY good, you did a great job, not just with the paper but the original work. I'm impressed, and honored to be associated with your work

[cid:image001.gif@01D593E6.0B371CA0]
David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• (b)(6) (assistants: Kimberly Barasch; Whitney Robinson)
• 301 496 4409
• (b)(6) <mailto:(b)(6)

[DMM Portrait B 12 29 15 P0923]

From: Ellen Carlin (b)(6)
Sent: Friday, November 1, 2019 9:33 AM
To: Morens, David (NIH/NIAID) [E] (b)(6)
Subject: Re:

Thanks so much for the rapid turn-around! All edits accepted. I think it's a nice piece. Billy is going to reach out to his editorial contact at The Lancet and see if he can get it invited.

I didn't realize the size of the (b)(6) until I visited (b)(6) a few years ago. They are indeed like (b)(6) . That story about the Edmund Fitzgerald would make an amazing book (though I'm sure it's already been written…).

Will keep you posted on the article. See you next week if you are coming to Cosmos Club!

Ellen

From: "Morens, David (NIH/NIAID) [E]" (b)(6) <mailto:(b)(6)
Date: Thursday, October 31, 2019 at 2:00 PM
To: Ellen Carlin (b)(6)
Subject: RE:

Ellen, WOW, this is wonderful, you've done a great job! Extremely well written and clear. I have no substantial comments, just a few tweak suggestions to tighten or clarify. They can be ignored if you think best.

Yes, (b)(6) is sort of a bulge in (b)(6), and the (b)(6) is nice, nicer than around (b)(6). The (b)(6) are huge, like oceans, and the (b)(6) and (b)(6). You may remember the 1970's hit song called (b)(6), which has since become a sort of modern folk classic. It's about the (b)(6)
(b)(6)

[cid:image003.gif@01D593E6.0B371CA0]
David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
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National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• (b)(6) (assistants: Kimberly Barasch; Whitney Robinson)
• 301 496 4409
• (b)(6) <mailto:(b)(6)

[DMM Portrait B 12 29 15 P0923]

From: Ellen Carlin (b)(6) <mailto:(b)(6)
Sent: Thursday, October 31, 2019 10:03 AM
To: Morens, David (NIH/NIAID) [E] (b)(6) <mailto:(b)(6)
Subject: Re:

Hi David! Well, you've definitely sold me. I thought of (b)(6)
(b)(6). I think (b)(6) will be right on (b)(6). I'll have a great excuse to visit now even if I skip (b)(6)!☺
Please find attached a draft of our proposed paper. Billy, Catherine, Franck, and Kanya and I have all been through it and it represents my best attempt at compromise of everyone's inputs. We need to keep the core text to 750 words. Please edit and change things as you see fit! If you do add anything more than a sentence, you'll need to find something comparable to cut. (I'm assuming Lancet is a stickler for word counts…)
Thanks again for being interested in partnering with us.

Ellen

From: "Morens, David (NIH/NIAID) [E]" (b)(6) <mailto: (b)(6)
Date: Friday, October 25, 2019 at 3:33 PM
To: Ellen Carlin (b)(6) <mailto: (b)(6)
Subject: RE:

There's one big ugly awful city – (b)(6) – and a few moderate sized towns, but most of the rest is pretty much the boondocks. (b)(6) means (b)(6), and even if you don't count the (b)(6) it is by far the wateriest place in the US. It's hard to be anywhere and drive a few miles in any direction without hitting a pond or small lake. Mostly just woods and water. Not a lot of people. (b)(6) (b)(6) and is maybe 50-100 miles long and a mile or less wide – and it is so beautiful…. All of the hundreds of times (b)(6) (b)(6), and I never recognized its beauty (b)(6).

OK, don't get me started.

By the way, (b)(6) is OK but sort of touristy. I think of it as (b)(6) Las Vegas, but without the gambling Lines of shops selling kitschy junk and souvenirs.

[cid:image005.gif@01D593E6.0B371CA0]
David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
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Bethesda, MD 20892-2520
• (b)(6) (assistants: Kimberly Barasch; Whitney Robinson)
• 301 496 4409
• (b)(6) <mailto: (b)(6)

[DMM Portrait B 12 29 15 P0923]

From: Ellen Carlin (b)(6) <mailto: (b)(6)
Sent: Friday, October 25, 2019 2:02 PM
To: Morens, David (NIH/NIAID) [E] (b)(6) <mailto: (b)(6)
Subject: Re:

Are you sure that's all (b)(6)? Some of it looked like the Caribbean to me! I honestly had no idea… I think most of us think of (b)(6) when we think of (b)(6). Honestly you've sold me! ((b)(6) has long been on my list, I just didn't know there was so much else to see…)

From: "Morens, David (NIH/NIAID) [E]" (b)(6) <mailto: (b)(6)
Date: Friday, October 25, 2019 at 1:04 PM
To: Ellen Carlin (b)(6) <mailto: (b)(6)

Subject: FW:

Ellen, you're gonna think I work for the tourism industry, but below is the email I sent to (b)(6) (b)(6). Take a look at some of the photos. In the middle is the (b)(6). I never found it very interesting (b)(6), but now I see what a beautiful place it really is….. Not selling tickets, but hoping to convince you of its charm!

[cid:image007.gif@01D593E6.0B371CA0]
David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
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National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• (b)(6) (assistants: Kimberly Barasch; Whitney Robinson)
• 301 496 4409
• (b)(6) <mailto:(b)(6)

[DMM Portrait B 12 29 15 P0923]

From: Morens, David (NIH/NIAID) [E]
Sent: Friday, March 1, 2019 1:27 PM
To: (b)(6) <mailto:(b)(6)
Cc: (b)(6) (b)(6) <mailto:(b)(6))
(b)(6) <mailto:(b)(6)
Subject:

Hi (b)(6), I hope you are still thinking of some time out in (b)(6) this summer.

Here's a few interesting pix to give you an idea of the place: for you AND (b)(6) !

Basically, except for a few big cities like (b)(6), just think, water, forests, lots of Wide open spaces with small towns here and there.

[cid:image046.jpg@01D5909D.8AF142F0]

(b)(6) has many ghost towns, like this one, (b)(6), an old mining town abandoned in the 1800s, and still sitting there just like it was. (b)(6)

[Image result for (b)(6)]

Lots of water and outdoor sports like kayaking

[Image result for (b)(6)]

Water, water, water everywhere, this is in the (b)(6)

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

(b)(6) at tulip time (gone long before summer, alas….)

[Image result for soo michigan]

The (b)(6) , where you can practically touch the boats, some over 1,000 long

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6) sites]

Pictured (b)(6) , along (b)(6) . The colors come from minerals like copper in the rocks. These are the famous (b)(6)

(b)(6)

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

Forests and wilderness everywhere

[Image result for hydroplane (b)(6)]

Hydroplane races are unforgettable experiences especially for kids. The boats can go close to 300 mph as they slip and slide over the water, and the sound of the engines is deafening. On the open straightaways the rooster tails (the spray they kick up) can be 80+ feet tall and, incredibly, over a mile long (that's how fast they are going: the planes are a mile away by the time

the spray falls back down to the water). Not for people with heart problems!

[Image result for (b)(6)]

Hundreds of lighthouses….

[Image result for (b)(6)]

(b)(6)

[Image result for ice sail boat]

Ice sailboats

[Image result for freighters (b)(6)]

A freighter on the (b)(6)

(b)(6)

[Image result for whitefish dinner (b)(6)]

The totally to-die-for (b)(6) whitefish, from up near the (b)(6) border….

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

(b)(6) today….

[Image result for (b)(6)]

(b)(6)

[Image result for (b)(6)]

(b)(6)

[Related image]

(b)(6), which are a kazillion square miles of sand

[Image result for (b)(6)]

[Related image]

[Image result for (b)(6)]

[Related image]

[Image result for (b)(6)]

[Image result for (b)(6)]

[Image result for (b)(6)]

[cid:image044.gif@01D5909D.8AF142F0]

David M. Morens, M.D.
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• 301 496 4409
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[DMM Portrait B 12 29 15 P0923]

From: Morens, David (NIH/NIAID) [E]
Sent: Fri, 6 Aug 2021 02:02:25 +0000
To: Jason Gale
Cc: (b)(6)
(b)(6) Garry, Robert F
Subject: Re: Chris Newman interview

Yes, and let's remember it is possible, maybe likely, to be infected but never sick. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 5, 2021, at 21:50, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

If I eventually become the 1000th journalist to write a book about SARS-CoV-2, this has got to make it in:

Chris Newman: *[00:29:10]* One thing he interestingly did tell us, and it was in our original paper but didn't make it into the scientific reports sort of sanitized version is that he (Xiao) knew these vendors very well. He would go and see them weekly. He was on first-name terms. They'd chat, have a cigarette and a drink together and so forth. None of them got sick. Not one of them got sick from coronavirus. So they were selling these animals, but they themselves didn't get it.

Am I right in thinking that a serosurvey of workers in the two wet markets in Guangdong implicated in the SARS outbreak found 30% had cross-reactive antibodies? Would be fasssssssscinating to know whether Wuhan's wildlife vendors had some level of immune protection from prior exposure to SARS-related coronaviruses.

JG

From: (b)(6) At: 08/06/21 09:57:55 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:), (b)(6)
Cc: (b)(6)
(b)(6)
Subject: Re: Chris Newman interview

Agree with Eddie. They tests to do with those blood samples depending on quantity, storage, and availability would be to look for antibodies to SARS-CoV-2 rather than looking for evidence of the virus itself. But yes, I can imagine that being difficult or impossible in the current climate.

Stephen

From: Edward Holmes (b)(6)
Sent: Thursday, August 5, 2021 5:55:20 PM
To: Jason Gale
Cc: (b)(6); Peter Daszak; (b)(6);
Wang Linfa; (b)(6); Stephen Goldstein
Subject: Re: Chris Newman interview

That's interesting Jason.

The blood samples could be very useful (depending on how they are stored) but they would to find a lab that is willing and able to look at them. Again, the politics could be tricky.

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T (b)(6)
E

On 6 Aug 2021, at 9:27 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Howdy,
I had a very interesting convo just now over Zoom with Chris Newman, the wildlife ecologist who worked on the Xiao paper in Scientific Reports. The publication's history is even more interesting than I thought. Couple of interesting things: the corresponding author Zhou was part of China's wildlife police/border control efforts (so knows a LOT!) and Xiao collected ticks from the wildlife he was surveying, so should have blood samples from infested animals from May 2017 until the market closure stopped data collection in Nov. 2019.

Jason

From: David Morens
Sent: Wed, 29 Sep 2021 10:46:23 -0400
To: Morens, David (NIH/NIAID) [E]
Subject: Fwd: Scientific American

David M. Morens, MD
(b)(6)
(b)(6)
(b)(6) (work)
(cell)

IMPORTANT: My gmail frequently sends incoming messages to Trash, which is apparently not correctable. If you don't hear from me in a reasonable time, please try again, call, or use my NIH email address

IMPORTANT: For US Government-related email, please also reply to my NIAID address

---------- Forwarded message ---------
From: Justin Ling (b)(6)
Date: Wed, Sep 29, 2021 at 10:12 AM
Subject: Scientific American
To: (b)(6)

Hi Dr. Morens,

Just following up on that interview request that Peter Daszak forwarded on a few weeks ago, regarding my piece on the utility/wisdom/security of Gain-of-Function research.

Had a great chat with Gerry Keusch last week. Would also be keen to talk to you, if you've got some time this week.

Justin Ling
Journalist
(b)(6)

From: Morens, David (NIH/NIAID) [E] [b6] [b6]
Sent: 8/15/2021 10:29:52 PM
To: Jason Gale [j.gale@bloomberg.net]
CC: [b6] Garry, Robert F [b6]; [b6]
Subject: RE: feedback on wild animals

I did not klnow he was in [b6]. Is he retired, I assume?

David

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
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Building 31, Room 7A-03
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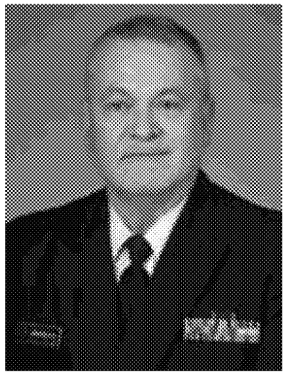
☎ [b6] (assistant: Whitney Robinson)
301 496 4409
[b6]



From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Sunday, August 15, 2021 6:29 PM
To: Morens, David (NIH/NIAID) [E] b6
Cc: b6; Garry, Robert F
b6
Subject: RE: feedback on wild animals

Be great to have you back in Australia, David. And of course, b6
b6 (known to virtually every virologist on this planet!) is in b6.

From: b6 At: 08/16/21 08:26:25 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: b6,
b6
Subject: RE: feedback on wild animals

Jason, not sure whose office politics is worse, but there is many a day I'd rather be in Australia, even if I had to drink that awful beer Fosters. (To quote Grouco Marx as he help up a glass of questionable beer: "last time I saw something like this, they had to shoot the horse")…. But I do have fond memories of Australia, and b6
b6, are out in b6, the only large city there (if oy can call it that) I have never been, although it's high on my bucket list if this damn pandemic ever ends. b6
b6.

David

David M. Morens, M.D.
CAPT, United States Public Health Service

Senior Advisor to the Director
Office of the Director
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National Institutes of Health

Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520

☎ b6 (assistant: Whitney Robinson)
301 496 4409
b6

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 7:54 PM
To: Morens, David (NIH/NIAID) [E] b6
Cc: b6 ; Garry, Robert F b6 ; b6
Subject: Re: feedback on wild animals

Thanks, David.

I just got off a video conf call with the current editor in NY (the one asking all the latest questions). He's very nice and I feel like he gets its. He thinks it's a fascinating story.

Problem we have in journalism is that there are some people who aren't interested in actual journalism and telling stories; they want to climb to the top and manage people. b6

[b6], but am so grateful that I still get to meet so many remarkable people, visit cool places and see all aspects of humanity.

A difficulty working for a NY-based organization is that it's assumed that all the best people are in NYC, so being in Melbourne (where I need to be to be [b6] [b6]), is that folks consider Australia a backwater and, by association, I mustn't be all that important/valuable. But my new role as "global biosecurity czar" is helping somewhat.

Ahhhhh... Office politics!

From: [b6] At: 08/15/21 09:03:03 UTC+10:00

To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: [b6],
[b6]
Subject: Re: feedback on wild animals

Jason, all I can say is that it must be harder to be a journalist than a scientist.... I had no idea someone at your level would get pushed back.

Usually i get from one to 4 reviewer responses to a ms. and three of those are out to lunch. Occasionally i get a reviewer who really understands the work: half of those are helpful, the other half are trashers.

Maybe it's like being in the government where i am: there is endless push back, but the push-backers are brainless idiots who don't know what they are talking about.

Do you ever get to a point where the editors leave you alone on the science? Or do they all think they are science geniuses? d

Sent from my iPhone

David M Morens

OD, NIAID, NIH

On Aug 14, 2021, at 16:05, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Thanks, Bob. In journalism, getting to the truth even when it's genuinely and actively pursued, can be a tortuous process!

----- Original Message -----
From: Robert F Garry b6
To: JASON GALE, b6,
b6
At: 08/15/21 01:45:11 UTC+10:00

Looking forward to this important article. Truth is stronger than fiction.

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 6:41 AM
To: b6
b6
; Garry, Robert F b6 ;
b6
Subject: Fwd:Re:feedback on wild animals

External Sender. Be aware of links, attachments and requests.

Hi guys, in case you have nothing better to read over the weekend, this is some of the dialog I am having with editors in the U.S. Essentially, my response to questions from editor #6.

I thought I could claim victory when it looked like the story

could be published this morning, but the b6 Businessweek objected and thinks b6 can make it better with another revision and an ETA of Tuesday. Sigh.

Jason

From: Jason Gale (BLOOMBERG/ NEWSROOM:) At: 08/14/21 16:36:58 UTC+10:00

To: Cristina Lindblad (BLOOMBERG/ NEWSROOM:) , Eric Gelman (BLOOMBERG/ NEWSROOM:)
Cc: Joel Weber (BLOOMBERG/ NEWSROOM:)
Subject: Re:feedback on wild animals

> Hi.
>
> Thanks for the feedback and the questions, which I have tried to answer in detail.
>
> It's often easier to have a conversation over Nexi or the phone to explain nuanced information, or details that are clear to me, but might not be to someone coming at this fresh, but that's tricky with the time difference.
>
> When you look back at what's happened here, it seems that in trying to deflect blame for the pandemic, which became increasingly vicious as the cataclysmic nature of the Covid-19 pandemic unfolded around March-

April 2020, China tried to conceal a very obvious, very plausible source of the pandemic: it's flourishing wildlife trade (worth about $90 billion in 2016).

It was an obvious cause of the pandemic because an almost identical scenario triggered an international outbreak caused by a very similar coronavirus (SARS) in 2003-04. But in attempting to cover up the wildlife trade, and making like there were never any wild animals being sold in Wuhan's wet markets, things began to backfire on China; questions were raised about the nearby lab studying these coronaviruses. The more geopolitical, heated and vicious the arguments and accusations became, the less cooperative China became. In response, the more intent/adamant some groups have become in their belief that China is covering up a lab-leak. It's become a vicious circle. If China isn't coming clean on the wild animals, what else is it trying to hide?? China's defensiveness means we may never get the cooperation needed to find the answers. If China had been honest and transparent about the wild animals in the

wet markets, it might not be in this mess now.

Anyway, here are my response to your queries in green.

By the way, I wonder if the current headline:

Delayed Paper Gives Credence to Wuhan Market Covid Origin Story

doesn't convey much more than what we had 2 months earlier when we reported Xiao's findings: China Markets Sold Mink, Civets, Stoking Natural Origins Theory

Perhaps Obscured China Paper Scuppered Chance to Trace Covid Origins

would hits "China", "Covid" and "Origins" in a way that won't alienate people who already believe it was a lab-leak

<><><><>

According to the report, minks, civets, raccoon dogs and other mammals known to harbor coronaviruses were sold in plain sight for years in shops across the city, including the now

infamous Huanan wet market, to which many of the earliest Covid cases were traced. The evidence collected over 30 months by **Xiao Xiao** a I'D NAME HIM HERE [[i don't know that naming a researcher no one has ever heard of adds much]] researcher working at a lab affiliated with China's Ministry of Education was hastily drafted into a manuscript and submitted to a scientific journal [Joel, we cannot name the first journal they delivered it to because the authors decline to give us the name, saying it may affect their future chances of being published] in February 2020, just weeks before the outbreak was declared a pandemic.

<><><><>

While the study received wide attention when it was eventually released by a different publisher [Publisher (Springer Nature) and publication (Scientific Reports) are different. Might add confusion DO YOU WANT TO ADD NAME HERE?], its long and torturous journey to publication gave **Chinese officials an opportunity to weave alternative narratives in which the virus may**

have come from abroad, even from a U.S. Army biological research facility. [the stuff in bold is not contested and we go on to show how that happened, documenting with links when available]I TWEAKED WORDING HERE BUT THIS STILL ISN'T QUITE RIGHT. IF THE REPORT HAD BEEN PUBLISHED EARLIER CHINESE OFFICIALS COULD STILL HAVE DONE WHAT THEY DID—THEY COULD HAVE JUST SAID THE REPORT WAS SHODDY, OR WHATEVER, AND THEY ALSO COULD HAVE JUST IGNORED IT. I disagree. The evidence that Xiao et al provide was meticulously documented and supported by photographs that would have been difficult/impossible for China to dismiss (as older published photos and media reports had been). What's more, Newman understands that Xiao collected blood-sucking ticks from the wild animals he studiously cataloged. His frozen tick samples could be tested for blood/antibodies/virus, which could be extremely helpful in identifying infected species PRIOR to December 2019. The WHO team knew nothing of this, so couldn't have asked China for this research or any results, had they actually done the research. The delay in the publication of Xiao's paper delayed the evidence that there were

live animals sold in the Huanan market. The WHO researchers couldn't have asked about tests on wildlife that ostensibly were never there. Likewise, Chinese authorities couldn't have done the tests on animals that didn't exist. The problem is that in January and February 2020, it was widely assumed the animals HAD been there and that the necessary tests and tracing of animals (the sampling of animals on farms they were raised on, testing of farm workers, animal hunters, transporters and traders **had all been done** by researchers in China -- the very things that ultimately led to the discovery of the origins of SARS and of MERS viruses. **None of these things were done** (or at least, there is nothing publicly available to show that they were done) **because the animals "weren't there"**. Now that we all know they were there, China as lost considerable face. The issue has become so political than there is much less/?no willingness to cooperate and conduct the additional research that the WHO-led team recommended. Ideally, Xiao's ticks should be studied, but it's doubtful that will now happen (I wouldn't be surprised if he's been

ordered to incinerate them!) Hope this is clear.

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An international team of experts convened by the WHO traveled to Wuhan earlier this year to seek answers—a trip that might have yielded different results if the scientists had known about the work of Xiao Xiao, a virologist whose roles straddled epidemiology and animal research at the government-funded Key Laboratory of Southwest China Wildlife Resources Conservation and at Hubei University of Traditional Chinese Medicine.'MIGHT HAVE YIELDED DIFFERENT RESULTS' SEEMS HIGHLY SPECULATIVE. WHAT RESULTS DID IT YIELD? I'D CUT THIS GRAF

As mentioned above, the WHO-convened team of researchers was told by market authorities, vendors and regular market visitors that there were no live animals sold in the Huanan market. That undermined completely the premise that the Huanan market was the kind of place where live animals from different species are stacked in

cages, with urine and fecal material drips from one cage to the one below it, where there are splatters of blood and guts from animals, and lots of potential for the spread of diseases from one species to another (including Homo sapiens). Instead, the market (and one other one known to have been selling live wild animals in 2019) was presented as selling only frozen wild animals and aquatic species and things unlikely to be the source of SARS-CoV-2. The WHO researchers were told there were frozen ferret badgers and other wildlife found in freezers. Some of the carcasses actually came from Yunnan, the province where the closest coronavirus related to SARS-CoV-2 was found in bats -- thus establishing a potential route from Yunnan to Wuhan in wildlife. That was actually important. When the researchers showed their Chinese counterparts photos of caged raccoon dogs taken in the Huanan market by Prof. Edward Holmes five or six years earlier, they were told by Chinese scientists that the photos may have been faked, and that the market had ceased selling such live animals anyway. The WHO researchers saw no

evidence (empty cages, animal pelts, etc) to dispute what the Chinese scientists told them, although they did smell "animals" -- but were told they were smelling rotten meat, sewage etc. Three people associated with the WHO-led mission told me that they didn't believe the information they were given by anyone associated with the market. But since there was no evidence to the contrary, they were unable to push the Chinese scientists further on this. **The fact that the mission concluded that frozen, not live wild animals were sold in the market undermined the thesis that Covid resulted from an animal spillover**. And the absence of strong evidence pointing to a spill over from wild animals to humans made the lab-leak theory look, in relative terms, more plausible.

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Six months and two revisions later, the journal's publishers rejected the paper. "They did not think it would have widespread appeal," says Newman, who declined to name the publication [do you want us to say why he won't name it? I don't think

it's necessary. There's no upside for scientists to make a publisher look bad (most journals are published by a handful of publishers)] "It caused us, especially our Chinese co-authors, concern that these data would not be taken seriously."

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The manuscript underwent a third revision to include data on China's pangolin trade networks (an earlier study, later contested, had implicated pangolins in the virus's spread to humans)WHO ASKED THAT THIS INFO BE INCLUDED? (I did, because we're trying to explain here why WHO missed the significance of Xiao's evidence. The WHO was slammed by other papers being submitted PLUS Xiao's paper had a weird title. That title seemed relevant to the authors back in January-February 2020, when pangolins were considered a possible SARS-CoV-2-spreading culprit. But in October 2020, pangolins were off the hook, so the title would have seem irrelevant/unimportant at first glance at WHO). It was then sent to the online journal *Scientific Reports*.

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The China-based researchers had reason to be cautious. In February 2020, the China Center for Disease Control (CCDC lets not use "CCDC". It's not commonly used like the U.S. "CDC" is, and it's going to force readers to go back and figure out what the "CCDC" is) prohibited scientists working on Covid-relate

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~~CCDC~~Disease detectives arriving from Beijing (think this makes it clear that these are China's disease "feds" arriving) on the first day of 2020 ordered environmental samples to be collected from drains and other surfaces at the market. Some 585 specimens were tested, of which 33 turned out to be positive for SARS-CoV-2. "All current evidence points to wild animals sold illegally," China CDC Director George Gao and colleagues wrote in the agency's weekly bulletin in late January. All but two of the positive specimens came from a cavernous and poorly-

ventilated section of the market's western wing, where many shops sold animals.

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~~The information void kindled a raging political debate that's already caused a trade war between China and Australia, as nations demand to know how Covid emerged.~~

Australia in April 2020 called for a global inquiry into the origins of the pandemic, including China's handling of the initial outbreak. Days later, then-U.S. Secretary of State Mike Pompeo used part of his Earth Day message to call on China to close its wet markets to "reduce risks to human health inside and outside of China."

Based on a discussion today with someone with knowledge of the WHO-led mission to Wuhan in Jan-Feb 2021, a significant catalyst for China's defensiveness is the emergence of **claims for reparations** that extended from the finger-pointing at China's wet markets. These surfaced in April 2020, and have continued

as recently as June 2021 (when Trump pushed for it again at a rally in the Midwest)

SO IS THIS THE POINT AT WHICH CHINA, WHICH SEEMINGLY ACCEPTED THE WET MARKET HYPOTHESIS, BEGAN TRYING TO CREATE A DIFFERENT NARRATIVE? (I think it was incremental. I believe China was embarrassed that its citizens were still buying wild animals in wet markets to eat -- a well-known hazard for zoonotic disease transmission that China tried unsuccessfully to outlaw almost 20 years ago. But that embarrassment/humiliation morphed into rigid denial and obfuscation when governments began openly blaming the Chinese Communist Party and agitating for China to pay reparations for the pandemic. See these clips:

- USA Today: Blame the Chinese Communist Party for the coronavirus crisis: Coronavirus crisis proves communism is still a grave threat to the entire world. If Beijing had just been honest, the pandemic could be

preventable. April 5, 2020

- Yahoo News: More than half of Americans think China should pay coronavirus reparations, poll shows April 9, 2020
- Voice of America: Americans Join Coronavirus Lawsuit to Make China Pay April 10, 2020
- Washington Post Opinion: China must pay reparations to Africa for its coronavirus failures April 16, 2020
- Reuters: In a first, Missouri sues China over coronavirus economic losses April 22, 2020
- Washington Post: Missouri is suing China over the coronavirus pandemic. It's the latest conservative gambit, April 22, 2020
- New York Post: Top German paper demands $165 billion coronavirus reparations from China April 22, 2020
- Attorney General Fitch Prepares to Sue China on Behalf of Mississippians April 22, 2020
- Newsweek: Trump on U.S. Seeking Compensation From China Over COVID-19: 'We Have Not

Determined the Final Amount' April 28, 2020

- The Guardian: Trump says China could have stopped Covid-19 and suggests US will seek damages April 28, 2020
- Intelligencer: Trump Thinks He Can Make China Pay for the Virus Like Mexico Paid for the Wall, April 30, 2020
- Washington Post: U.S. officials crafting retaliatory actions against China over coronavirus as President Trump fumes April 30, 2020
- Lawfare: Does China Really Owe the World Trillions of Dollars? May 7, 2020
- Fortune: Trump's demand that China pay coronavirus reparations evokes an ugly history May 8, 2020
- South China Morning Post: Why China won't be paying the West coronavirus reparations any time soon May 15, 2020
- Deccan Herald, India: Abhijit Bhattacharyya | Why China needs to pay reparations to the world June 4, 2020
- Why calls for reparations from China for coronavirus are an

unfeasible distraction June 9, 2020

- Newsweek: Trump Demands China 'Pay Reparations' for COVID, Says $10 Trillion Not Enough June 12, 2021

In response, Geng Shuang, a spokesman for China's Foreign Ministry, denied "wildlife wet markets" existed in the country. Government researchersCAN WE BE MORE SPECIFIC? Twenty-two researchers from mostly nationally-funded laboratories (I think it's the Chinese equivalent of the NIH in the U.S.) and institutes attached to the Chinese Academy of Sciences) now dismiss the market hypothesis completely. "SARS-CoV-2 could not have possibly evolved in an animal market in a big city and even less likely in a laboratory," they wrote in a paper released last month ahead of publicationWHERE/WHEN WILL IT BE PUBLISHED? It was released as a "pre-print" on a Chinese academic repository ahead of publication that appears to be managed/owned by the Chinese Academy of Sciences. Papers are usually released in pre-print form before they have been accepted for

publication or peer-reviewed as a way of expediting public access to the information. In this case, there is no information to suggest if, where or when the paper will be published.

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A more recent paperBY WHOM? (China CDC's Gao and eight other scientists mostly from the Chinese of Academy of Sciences' institutes) contends that the virus may have been imported from multiple locations worldwide, including parts of Europe where mink are raised in areas inhabited also by horseshoe bats known to harbor coronaviruses. "The official narrative changed not because the evidence changed," says Robert Garry, a professor of microbiology and immunology at Tulane University's School of Medicine in New Orleans "A spillover from a wet market was what caused SARS, and, embarrassingly for China, those wet markets were never shut down." Garry is the co-author of one of the earliest papers on the origins of Covid but wasn't involved in the research on Wuhan's markets.

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I DON'T REALLY UNDERSTAND WHAT POINT THIS GRAF IS TRYING TO MAKE. This is intended to demonstrate the kind of gaslighting that has occurred. Some of the WHO-led researchers are veterinarians and zoologists -- they know what animals smell like, and could smell their lingering presence a year later, but were told essentially that it was impossible that they were smelling animals because **there "were no live animals there**".) The researchers noted a mixed smell of animals and disinfectant in some areas of the market, but they were told by the market's manager that they were probably smelling the lingering stench of rotten meat and sewage, according to a joint WHO-China report. [should we add "according to Laing Wannian etc here? Liang Wannian was the leader of the Chinese research team collaborating jointly with the WHO-led research team. The source for the above description are in the annexes to the official joint WHO-China report released at the end of March 2021.]

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Earlier the same day, the international research team visited Wuhan's larger Baishazhou market, where Xiao had regularly surveyed two sellers of live wild animals. Yet the group was told only frozen food, ingredients, and kitchenware were on offer there. Liang Wannian, an epidemiologist who led the Chinese experts collaborating with the WHO-convened team, says his group had no knowledge of Xiao's data either. [if the above information on what the delegation saw all comes from this same source, maybe we should include his name higher up (The source for the description of what the origins researchers saw on Jan. 31, 2021, comes from the official120-page joint WHO-China report and its193-page annexes, not from Liang. Since we are essentially accusing China of concealing the information that Xiao documented, we asked Liang at a press conference in late July 2021 when the Chinese team first knew about Xiao's findings -- that the Huanan market and three others in Wuhan were selling live animals permissive to

SARS-CoV-2 infection -- and what research China has done subsequently as a follow-up on this information? Liang gave a very long-winded response in Chinese in which he said essentially "we didn't have that information in January-February 2021 when the research team was in Wuhan". I think it's important we keep this to demonstrate that we have tried to ascertain what China knew/has done and have given the Chinese researchers the opportunity to respond. In addition, I have emailed China CDC Director George Gao at least twice and not received any response.]

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Among the earliest clusters of infections recorded in Wuhan, one involved three Covid cases among staff working at a stall in Huanan. One of the employees, a 32-year-old who fell ill on Dec. 19, traded goods back and forth between the Huanan and Baishazhou markets.

WHERE DOES THIS FACT COME FROM? NOT THE REPORT, RIGHT? WHEN DID IT FIRST BECOME KNOWN? This information was from the joint WHO-China

report released in March 2021, however, the WHO-led researchers went to the Baishazhou market more as a demonstration by the China team of what a perfectly functioning large food market looks like. The WHO-led team was oblivious to Xiao's research that showed there were at least two stalls in Baishazhou that had been selling live wild animals for human consuption. So this detail and its significance was lost on the WHO researchers at the time.)

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A confirmed case linking two markets that sold wild animals is "very intriguing,"says Stephen Goldstein, a research associate in evolutionary virology at the University of Utah in Salt Lake City. But tracing any contact the employee might have had with infected wildlife is impossible now that the animals are long gone.WOULD TIMELY PUBLICATION OF THE REPORT HAVE CHANGED THIS? It's unlikely that Xiao et al's paper would have been *published* soon after It was drafted in February 2020, but it could have been *released* as a pre-print ahead of

publication and peer-review that same month. That would have **confirmed** what almost everyone had suspected: that there **was** a flourishing wildlife trade in Wuhan that provides a plausible pathway by which coronavirus-infected wildlife from Yunnan and beyond could have introduced the virus to the city, sparking the Covid outbreak. It's also possible that swift recognition of these potential wild-animal vectors could have allowed scientists to test them for the virus and for antibodies against the virus while they were still alive (perhaps not the ones from Huanan, but wildlife in the three other Wuhan markets). Because of the statements the Chinese authorities had been making in January and February that strongly supported the animal spill over theory, it was assumed that this research was being done. Of course, the pandemic was raging then, so it would have been challenging. But, because of the denial by Chinese researchers that wild animals were being sold in Wuhan wet markets before the pandemic, there was never that level of follow up. At least, if there was, it was never made public.)

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“It seems to me, at a minimum, that local or regional authorities kept that informationWHAT INFORMATION? (that Wuhan had a flourishing live wild animal business going on in its wet markets before the pandemic) quiet deliberately,” Goldstein says. “It’s incredible to me that people theorize about one type of cover-up, but an obvious cover-up is staring them right in the face.”

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From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 8/21/2021 12:13:32 AM
To: Jason Gale [j.gale@bloomberg.net]
CC: b6 Garry, Robert F [b6]; b6
BCC: Morens, David (NIH/NIAID) [E] [b6 b6]
Subject: Re:

Good names. b6 has a certain paranoid streak though…. I might add b6 from b6, the phylogeneticist b6 (kinda hard assed but honest and detailed), b6 (elder statesman with strong international experience including smallpox erad and ebola discovery, b6 from b6, b6 would be great!, b6 the bat epidemiologist/epizootiologist, b6 in b6 and many more!
d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 20, 2021, at 20:02, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I am big on diversity and think middle-aged white men (of which I am one) are usually over-represented, so I'd like to see a good array of very talented smart women considered...the likes of:
*
*
*
*
*
*

b6

From: b6 At: 08/21/21 09:54:52 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: b6

b6

Subject: Re:

Yes, i did see this, but assume it is all rigged. Can this group ID some folks who would be good candidates???? I can think of a few names…. d

Sent from my iPhone
David M Morens

OD, NIAID, NIH

On Aug 20, 2021, at 19:30, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Meantime, y'all saw this, right?
https://www.who.int/news-room/articles-detail/who-scientific-advisory-group-for-the-origins-of-novel-pathogens-(sago)

From: b6 At: 08/21/21 09:27:14 UTC+10:00
To: b6
Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) , b6
b6
Subject: Re:

Those Italian sequences are stone cold contamination David. Nothing nefarious, just a poorly done study.

The following Tweet threat by Michael Worobey explains it beautifully:

https://twitter.com/MichaelWorobey/status/1424483875384958981?s=20

Cheers,

Eddie

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6

On 21 Aug 2021, at 9:10 am, Morens, David (NIH/NIAID) [E] b6 wrote:

Eddie, thanks so much, I had no idea that some of these conflicting data represented bullshit agendas. What has happened to scientific integrity that scientists would sell their souls over dishonest political agendas? I guess i am too naïve…. I have always believed or at least hoped that scientists had the utmost integrity….

If i may impose on you again, last week the Italian group published, finally, their data on viral sequences dating back to early-mid October 2019 and thereafter from Italy, suggesting, or so the data seem to say, that their sequences are upstream of the earliest Wuhan sequences two months later.

If true, this would suggest an earlier viral origin spread to Europe before being detected in Wuhan. The Italian sequences seemed to suggest that the Wuhan virus was a downstream offshoot?

Perhaps I misunderstand, either that or the authors are nuts? Surely you guys can figure this out? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 20, 2021, at 17:25, Edward Holmes b6 wrote:

It's diabolical nonsense David. Irrespective of what they state in that 'paper', Linfa has found serological evidence for closely related viruses in pangolins dating back several years and the HKU team have similar data (see attachment). Plus the Guangdong pangolins have been my multiple groups in different ways and there is an independent lineage in Guangxi.

The attempt to undermine the pangolin data and the people that generated it one of the shameful examples of anti-science I have ever seen. The reality is that is because the RBD of the Guangdong pangolins is genetically similar to SARS-CoV-2 it becomes an inconvenient data point for those who believe the virus came from a lab in Wuhan hence their attempts to undermine it.

Cheers,

Eddie

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6 <mailto: b6

On 21 Aug 2021, at 1:03 am, Morens, David (NIH/NIAID) [E]
b6 <mailto: b6 >> wrote:

Thanks to both you and Kristian. Very heelpful to know what the experts think, because 5o us mere

mortals, phylogenetic and sequencing interpretation is a bit inscrutable.

Yes, although I don't know her personally, I know OF Alina Chan based on two papers of hers I came across, one of which was a screed against Eddie's recent review. It seemed biased, cherry-picked, and not the work of a scientist with integrity.

<image004.gif>

David M. Morens, M.D.
CAPT, United States Public Health Service
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National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• b6 (assistant: Whitney Robinson)
• 301 496 4409
• b6 <mailto: b6 >

<image005.jpg>

From: Garry, Robert F b6 <mailto: b6 >>
Sent: Friday, August 20, 2021 10:38 AM
To: Morens, David (NIH/NIAID) [E] b6 <mailto: b6 >>;
Kristian G. Andersen b6 <mailto: b6 >>
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>;
b6 <mailto: b6 >;
b6 <mailto: b6 ;
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b6 <mailto: b6 >
Subject: Re:

David,

This from a really super young investigator Alex Crits-Christoph. The authors concluded:

“(a) the pangolin covs are actually from mice (b) actually, they were actually cloned artificial constructs, (c) actually, there were other viruses in the samples as well (oh no! who'd have thought), (d) actually, it's all contaminated with dog dna.”

My take: It is garbage and no they [the authors] are not ok - although my supposition is that they are being well compensated for generating this nonsense. Alina Chan [who is a quite dangerous IMO young investigator and is writing a book] is using the very same approach - spouting a lot of pseudoscientific garbage, arguing from "authority." etc., but finding a receptive [and likely wealthy] audience that can put the garbage to work. The whole Dr. Yan/Steve Bannon saga is but one of the examples of this approach.

b

From: "Morens, David (NIH/NIAID) [E]" b6 <mailto: b6 >>
Date: Friday, August 20, 2021 at 8:56 AM
To: Kristian Andersen b6 <mailto: b6 >>
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>,
" b6 <mailto: b6 >"
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" b6 <mailto: b6 >"
b6 <mailto: b6 >>, Robert Garry
b6 <mailto: b6 >>,
" b6 <mailto: b6 >"
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" b6 <mailto: b6 >"
b6 <mailto: b6 >>
Subject: <no subject>

External Sender. Be aware of links, attachments and requests.
Do you all know these data? see link below….
[2108.08163] Cloning vectors and contamination in metagenomic datasets raise concerns over pangolin CoV genome authenticity (arxiv.org)<https://protect-au.mimecast.com/s/s7cRCQnMBZfkxWRNQTxp1ID?domain=nam11.safelinks.protection.outlook.com>

<image006.gif>

David M. Morens, M.D.
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Senior Advisor to the Director
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National Institutes of Health
Building 31, Room 7A-03

31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• b6 (assistant: Whitney Robinson)
• 301 496 4409
• b6 <mailto: b6

<image007.jpg>

From: Kristian G. Andersen b6 <mailto: b6 >>
Sent: Thursday, August 12, 2021 8:11 PM
To: Morens, David (NIH/NIAID) [E] b6 <mailto: b6 >>
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 >; b6
b6 <mailto: b6 >;
b6 <mailto: b6 >; Garry, Robert F
b6 <mailto: b6 >>;
b6 <mailto: b6 ;
b6 <mailto: b6
Subject: Re: The story behind the missing story about the story behind the missing raccoons

I hear La Jolla has some pretty nice beaches - just saying.

Oh wait, I live here - here's what's outside my office:

<image008.jpg>

Happy to save you a spot - you know, 'field' research.

K

On Thu, Aug 12, 2021 at 5:09 PM Morens, David (NIH/NIAID) [E]
b6 <mailto: b6 >> wrote:

You deserve that beach! Reminds me of that Warren Zevon song about “sippin’ Fosters in the shade”…. Mr. Bad example, i think it was…. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 12, 2021, at 20:00, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>> wrote:

Thanks, David. I've actually been tied up with a podcast series on long Covid (while trying to stay on top of the usual vaccine effectiveness stuff. Busyness with which y'all are only too familiar!). But it helps to vent sometimes about you can feel pretty defeated by your job. Thanks for the support. There will be a beach for me to lay on somewhere some day... JG

From: b6 <mailto: b6 > At: 08/13/21 09:05:19 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:) <mailto:j.gale@bloomberg.net> ,
b6 <mailto: b6 <mailto:
b6 <mailto: b6 ,
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b6 <mailto: b6
Subject: RE: The story behind the missing story about the story behind the missing raccoons

Jason, yikes!, but it is a miracle that with all that work you have still been able to crank out multiple high-calibre articles. I have no idea why anyone up your chanin would jerk you around. Who are these guys anyway???? Just keep doing it and overcome, OK?

<image006.gif>

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
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Bethesda, MD 20892-2520
• b6 (assistant: Whitney Robinson)
•301 496 4409
• b6 <mailto: b6 >

<image007.jpg>

From: Jason Gale (BLOOMBERG/ NEWSROOM:)
<j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>
Sent: Thursday, August 12, 2021 5:53 PM
To: b6 <mailto: b6 >;
b6 <mailto: b6 >;
b6 <mailto: b6 >; Morens, David (NIH/NIAID)
[E] b6 <mailto: b6 >>;
b6 <mailto: b6
b6 <mailto: b6 >; b6 <mailto: b6 >;
Garry, Robert F
b6 <mailto: b6 <mailto: b6
b6 <mailto: b6
Subject: The story behind the missing story about the story behind the missing raccoons

Hi everyone,
Just letting you know that my story has been turned into a sh!tshow internally. My long awaited feature on why the raccoon dogs were there in Wuhan one minute, gone the next and why we waited 18 months to find out for sure that they were there in the first place, has taken more twists and turns than any Olympic diver, thanks to some egomaniac editors. (Please keep that bit to yourselves).
I have even more sympathy for Xiao et al. I'm told now Tuesday for publication, but I wouldn't be surprised if some a-hole higher up the food chain spikes it. To say I am exasperated (and a tad emotional after working 13 days straight) is an understatement.
Kindest regards,
Jason

<Pangolin-Serology-Nido2021-Poster.pdf>

From: Peter Daszak [b6]
Sent: 8/15/2021 6:06:31 PM
To: Jason Gale [j.gale@bloomberg.net]; Morens, David (NIH/NIAID) [E] [b6]
Group ([b6]
CC: [b6]; Garry, Robert F [/o=ExchangeLabs/ou=Exchange
Administrative Group ([b6]); [b6]
Subject: RE: feedback on wild animals

Disappointing your editor doesn't get it yet.

All off-the-record, but please talk to your editor about how hard we argued with the China side re. whether live mammals were being sold or not. The fact that we couldn't state categorically in the WHO report that live mammals of species known to host SARSr-CoVs were present in the market was used by the lab leak contingent to ridicule the evidence we did find – frozen ferret-badger & rabbit carcasses left behind after the cleanout. Anyone with half a brain could see what was going on here, but having that paper as evidence would have built a much stronger case in my opinion.

Also mention to your editors that the truth will out – there are papers coming down the pipeline that add weight to a bat-int.host-human pathway and zero for the lab leak BS. This article will be one of the few right now that gets on the right side of history – surely that matters?

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
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USA

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 7:54 PM
To: [b6]
Cc: [b6];
[b6]
Subject: Re: feedback on wild animals

Thanks, David.

I just got off a video conf call with the current editor in NY (the one asking all the latest questions). He's very nice and I feel like he gets its. He thinks it's a fascinating story.
Problem we have in journalism is that there are some people who aren't interested in actual journalism and telling stories; they want to climb to the top and manage people. I [b6], but am so grateful that I still get to meet so many remarkable people, visit cool places and see all aspects of humanity.
A difficulty working for a NY-based organization is that it's assumed that all the best people are in NYC, so being in Melbourne ([b6] [b6]), is that folks consider Australia a backwater and, by association, I mustn't be all that important/valuable. But my new role as "global biosecurity czar" is helping somewhat. Ahhhhh... Office politics!

From: [b6] At: 08/15/21 09:03:03 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: [b6], [b6]
Subject: Re: feedback on wild animals

Jason, all I can say is that it must be harder to be a journalist than a scientist…. I had no idea someone at your level would get pushed back.

Usually i get from one to 4 reviewer responses to a ms. and three of those are out to lunch. Occasionally i get a reviewer who really understands the work: half of those are helpful, the other half are trashers.

Maybe it's like being in the government where i am: there is endless push back, but the push-backers are brainless idiots who don't know what they are talking about.

Do you ever get to a point where the editors leave you alone on the science? Or do they all think they are science geniuses? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 14, 2021, at 16:05, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Thanks, Bob. In journalism, getting to the truth even when it's genuinely and actively pursued, can be a tortuous process!

----- Original Message -----
From: Robert F Garry [b6]
To: JASON GALE, [b6],
[b6],

b6

At: 08/15/21 01:45:11 UTC+10:00

Looking forward to this important article. Truth is stronger than fiction.

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 6:41 AM
To: b6

b6

Garry, Robert F b6

b6

Subject: Fwd:Re:feedback on wild animals

External Sender. Be aware of links, attachments and requests.

Hi guys, in case you have nothing better to read over the weekend, this is some of the dialog I am having with editors in the U.S. Essentially, my response to questions from editor #6.
I thought I could claim victory when it looked like the story could be published this morning, but b6 of Businessweek objected and thinks b6 can make it better with another revision and an ETA of Tuesday.
Sigh.
Jason

From: Jason Gale (BLOOMBERG/ NEWSROOM:) At: 08/14/21 16:36:58 UTC+10:00
To: Cristina Lindblad (BLOOMBERG/ NEWSROOM:) , Eric Gelman (BLOOMBERG/ NEWSROOM:)
Cc: Joel Weber (BLOOMBERG/ NEWSROOM:)
Subject: Re:feedback on wild animals

> Hi.
> Thanks for the feedback and the questions, which I have tried to answer in detail.
> It's often easier to have a conversation over Nexi or the phone to explain nuanced information, or details that are clear to me, but might not be to someone coming at this fresh, but that's tricky with the time difference.

When you look back at what's happened here, it seems that in trying to deflect blame for the pandemic, which became increasingly vicious as the cataclysmic nature of the Covid-19 pandemic unfolded around March-April 2020, China tried to conceal a very obvious, very plausible source of the pandemic: it's flourishing wildlife trade (worth about $90 billion in 2016).
It was an obvious cause of the pandemic because an almost identical scenario triggered an international outbreak caused by a very similar coronavirus (SARS) in 2003-04. But in attempting to cover up the wildlife trade, and making like there were never any wild animals being sold in Wuhan's wet markets, things began to backfire on China; questions were raised about the nearby lab studying these coronaviruses. The more geopolitical, heated and vicious the arguments and accusations became, the less cooperative China became. In response, the more intent/adamant some groups have become in their belief that China is covering up a lab-leak. It's become a vicious circle. If China isn't coming clean on the wild animals, what else is it trying to hide??
China's defensiveness means we may never get the cooperation needed to find the answers. If China had been honest and transparent about the wild animals in the wet markets, it might not be in this mess now.

Anyway, here are my response to your queries in green.

By the way, I wonder if the current headline:
Delayed Paper Gives Credence to Wuhan Market Covid Origin Story

doesn't convey much more than what we had 2 months earlier when we reported Xiao's findings: China Markets Sold Mink, Civets, Stoking Natural Origins Theory
Perhaps Obscured China Paper Scuppered Chance to Trace Covid Origins
would hits "China", "Covid" and "Origins" in a way that won't alienate people who already believe it was a lab-leak

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According to the report, minks, civets, raccoon dogs and other mammals known to harbor coronaviruses were sold in plain sight for years in shops across the city, including the now infamous Huanan wet market, to which many of the earliest Covid cases were traced. The evidence collected over 30 months by **Xiao Xiao** a I'D NAME HIM HERE [[i don't know that naming a researcher no one has ever heard of adds much]] researcher working at a lab affiliated with China's Ministry of Education was hastily drafted into a manuscript and submitted to a scientific journal [Joel, we cannot name the first journal they delivered it to because the authors decline to give us the name, saying it may affect their future chances of being published] in February 2020, just weeks before the outbreak was declared a pandemic.

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While the study received wide attention when it was eventually released by a different publisher [Publisher (Springer Nature) and publication (Scientific Reports) are different. Might add confusion DO YOU WANT TO ADD NAME HERE?], its long and torturous journey to

publication gave **Chinese officials an opportunity to weave alternative narratives in which the virus may have come from abroad, even from a U.S. Army biological research facility.** [the stuff in bold is not contested and we go on to show how that happened, documenting with links when available]I TWEAKED WORDING HERE BUT THIS STILL ISN'T QUITE RIGHT. IF THE REPORT HAD BEEN PUBLISHED EARLIER CHINESE OFFICIALS COULD STILL HAVE DONE WHAT THEY DID—THEY COULD HAVE JUST SAID THE REPORT WAS SHODDY, OR WHATEVER, AND THEY ALSO COULD HAVE JUST IGNORED IT. I disagree. The evidence that Xiao et al provide was meticulously documented and supported by photographs that would have been difficult/impossible for China to dismiss (as older published photos and media reports had been). What's more, Newman understands that Xiao collected blood-sucking ticks from the wild animals he studiously cataloged. His frozen tick samples could be tested for blood/antibodies/virus, which could be extremely helpful in identifying infected species PRIOR to December 2019. The WHO team knew nothing of this, so couldn't have asked China for this research or any results, had they actually done the research. The delay in the publication of Xiao's paper delayed the evidence that there were live animals sold in the Huanan market. The WHO researchers couldn't have asked about tests on wildlife that ostensibly were never there. Likewise, Chinese authorities couldn't have done the tests on animals that didn't exist. The problem is that in January and February 2020, it was widely assumed the animals HAD been there and that the necessary tests and tracing of animals (the

sampling of animals on farms they were raised on, testing of farm workers, animal hunters, transporters and traders **had all been done** by researchers in China -- the very things that ultimately led to the discovery of the origins of SARS and of MERS viruses. **None of these things were done** (or at least, there is nothing publicly available to show that they were done) **because the animals "weren't there"**. Now that we all know they were there, China as lost considerable face. The issue has become so political than there is much less/?no willingness to cooperate and conduct the additional research that the WHO-led team recommended. Ideally, Xiao's ticks should be studied, but it's doubtful that will now happen (I wouldn't be surprised if he's been ordered to incinerate them!) Hope this is clear.

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An international team of experts convened by the WHO traveled to Wuhan earlier this year to seek answers—a trip that might have yielded different results if the scientists had known about the work of Xiao Xiao, a virologist whose roles straddled epidemiology and animal research at the government-funded Key Laboratory of Southwest China Wildlife Resources Conservation and at Hubei University of Traditional Chinese Medicine.'MIGHT HAVE YIELDED DIFFERENT RESULTS' SEEMS HIGHLY SPECULATIVE. WHAT RESULTS DID IT YIELD? I'D CUT THIS GRAF
As mentioned above, the WHO-convened team of researchers was told by market authorities, vendors and regular market visitors that there were no live animals sold in the Huanan market. That undermined completely the

premise that the Huanan market was the kind of place where live animals from different species are stacked in cages, with urine and fecal material drips from one cage to the one below it, where there are splatters of blood and guts from animals, and lots of potential for the spread of diseases from one species to another (including Homo sapiens). Instead, the market (and one other one known to have been selling live wild animals in 2019) was presented as selling only frozen wild animals and aquatic species and things unlikely to be the source of SARS-CoV-2. The WHO researchers were told there were frozen ferret badgers and other wildlife found in freezers. Some of the carcasses actually came from Yunnan, the province where the closest coronavirus related to SARS-CoV-2 was found in bats -- thus establishing a potential route from Yunnan to Wuhan in wildlife. That was actually important. When the researchers showed their Chinese counterparts photos of caged raccoon dogs taken in the Huanan market by Prof. Edward Holmes five or six years earlier, they were told by Chinese scientists that the photos may have been faked, and that the market had ceased selling such live animals anyway. The WHO researchers saw no evidence (empty cages, animal pelts, etc) to dispute what the Chinese scientists told them, although they did smell "animals" -- but were told they were smelling rotten meat, sewage etc. Three people associated with the WHO-led mission told me that they didn't believe the information they were given by anyone associated with the market. But since there was no evidence to the contrary, they were unable to push the Chinese

scientists further on this. **The fact that the mission concluded that frozen, not live wild animals were sold in the market undermined the thesis that Covid resulted from an animal spillover.** And the absence of strong evidence pointing to a spill over from wild animals to humans made the lab-leak theory look, in relative terms, more plausible.

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Six months and two revisions later, the journal's publishers rejected the paper. "They did not think it would have widespread appeal," says Newman, who declined to name the publication [do you want us to say why he won't name it? I don't think it's necessary. There's no upside for scientists to make a publisher look bad (most journals are published by a handful of publishers)] "It caused us, especially our Chinese co-authors, concern that these data would not be taken seriously."

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The manuscript underwent a third revision to include data on China's pangolin trade networks (an earlier study, later contested, had implicated pangolins in the virus's spread to humans)WHO ASKED THAT THIS INFO BE INCLUDED? (I did, because we're trying to explain here why WHO missed the significance of Xiao's evidence. The WHO was slammed by other papers being submitted PLUS Xiao's paper had a weird title. That title seemed relevant to the authors back in January-February 2020, when pangolins were considered a possible SARS-CoV-2-spreading culprit. But in October 2020, pangolins were off the hook, so the title would have seem

irrelevant/unimportant at first glance at WHO). It was then sent to the online journal *Scientific Reports*.

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The China-based researchers had reason to be cautious. In February 2020, the China Center for Disease Control (CCDC lets not use "CCDC". It's not commonly used like the U.S. "CDC" is, and it's going to force readers to go back and figure out what the "CCDC" is) prohibited scientists working on Covid-relate

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~~CCDC~~ Disease detectives arriving from Beijing (think this makes it clear that these are China's disease "feds" arriving) on the first day of 2020 ordered environmental samples to be collected from drains and other surfaces at the market. Some 585 specimens were tested, of which 33 turned out to be positive for SARS-CoV-2. "All current evidence points to wild animals sold illegally," China CDC Director George Gao and colleagues wrote in the agency's weekly bulletin in late January. All but two of the positive specimens came from a cavernous and poorly-ventilated section of the market's western wing, where many shops sold animals.

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~~The information void kindled a raging political debate that's already caused a trade war between China and Australia, as nations demand to know how Covid emerged.~~ Australia in April 2020 called for a global inquiry into the origins of the pandemic, including China's

handling of the initial outbreak. Days later, then-U.S. Secretary of State Mike Pompeo used part of his Earth Day message to call on China to close its wet markets to "reduce risks to human health inside and outside of China."

Based on a discussion today with someone with knowledge of the WHO-led mission to Wuhan in Jan-Feb 2021, a significant catalyst for China's defensiveness is the emergence of **claims for reparations** that extended from the finger-pointing at China's wet markets. These surfaced in April 2020, and have continued as recently as June 2021 (when Trump pushed for it again at a rally in the Midwest)

SO IS THIS THE POINT AT WHICH CHINA, WHICH SEEMINGLY ACCEPTED THE WET MARKET HYPOTHESIS, BEGAN TRYING TO CREATE A DIFFERENT NARRATIVE? (I think it was incremental. I believe China was embarrassed that its citizens were still buying wild animals in wet markets to eat -- a well-known hazard for zoonotic disease transmission that China tried unsuccessfully to outlaw almost 20 years ago. But that embarrassment/humiliation morphed into rigid denial and obfuscation when governments began openly blaming the Chinese Communist Party and agitating for China to pay reparations for the pandemic. See these clips:

- USA Today: Blame the Chinese Communist Party for the coronavirus crisis: Coronavirus crisis proves communism is still a grave threat to the entire world. If Beijing had just been honest, the pandemic could be preventable. April 5, 2020

- Yahoo News: More than half of Americans think China should pay coronavirus reparations, poll shows April 9, 2020
- Voice of America: Americans Join Coronavirus Lawsuit to Make China Pay April 10, 2020
- Washington Post Opinion: China must pay reparations to Africa for its coronavirus failures April 16, 2020
- Reuters: In a first, Missouri sues China over coronavirus economic losses April 22, 2020
- Washington Post: Missouri is suing China over the coronavirus pandemic. It's the latest conservative gambit, April 22, 2020
- New York Post: Top German paper demands $165 billion coronavirus reparations from China April 22, 2020
- Attorney General Fitch Prepares to Sue China on Behalf of Mississippians April 22, 2020
- Newsweek: Trump on U.S. Seeking Compensation From China Over COVID-19: 'We Have Not Determined the Final Amount' April 28, 2020
- The Guardian: Trump says China could have stopped Covid-19 and suggests US will seek damages April 28, 2020
- Intelligencer: Trump Thinks He Can Make China Pay for the Virus Like Mexico Paid for the Wall, April 30, 2020
- Washington Post: U.S. officials crafting retaliatory actions against China over coronavirus as President Trump fumes April 30, 2020
- Lawfare: Does China Really Owe the World Trillions of Dollars? May 7, 2020
- Fortune: Trump's demand that China pay coronavirus

reparations evokes an ugly history May 8, 2020
- South China Morning Post: Why China won't be paying the West coronavirus reparations any time soon May 15, 2020
- Deccan Herald, India: Abhijit Bhattacharyya | Why China needs to pay reparations to the world June 4, 2020
- Why calls for reparations from China for coronavirus are an unfeasible distraction June 9, 2020
- Newsweek: Trump Demands China 'Pay Reparations' for COVID, Says $10 Trillion Not Enough June 12, 2021

In response, Geng Shuang, a spokesman for China's Foreign Ministry, denied "wildlife wet markets" existed in the country. Government researchersCAN WE BE MORE SPECIFIC? Twenty-two researchers from mostly nationally-funded laboratories (I think it's the Chinese equivalent of the NIH in the U.S.) and institutes attached to the Chinese Academy of Sciences) now dismiss the market hypothesis completely. "SARS-CoV-2 could not have possibly evolved in an animal market in a big city and even less likely in a laboratory," they wrote in a paper released last month ahead of publicationWHERE/WHEN WILL IT BE PUBLISHED? It was released as a "pre-print" on a Chinese academic repository ahead of publication that appears to be managed/owned by the Chinese Academy of Sciences. Papers are usually released in pre-print form before they have been accepted for publication or peer-reviewed as a way of expediting public access to the information. In this case, there is no information to suggest

if, where or when the paper will be published.

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A more recent paperBY WHOM? (China CDC's Gao and eight other scientists mostly from the Chinese of Academy of Sciences' institutes) contends that the virus may have been imported from multiple locations worldwide, including parts of Europe where mink are raised in areas inhabited also by horseshoe bats known to harbor coronaviruses. "The official narrative changed not because the evidence changed," says Robert Garry, a professor of microbiology and immunology at Tulane University's School of Medicine in New Orleans "A spillover from a wet market was what caused SARS, and, embarrassingly for China, those wet markets were never shut down." Garry is the co-author of one of the earliest papers on the origins of Covid but wasn't involved in the research on Wuhan's markets.

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I DON'T REALLY UNDERSTAND WHAT POINT THIS GRAF IS TRYING TO MAKE. This is intended to demonstrate the kind of gaslighting that has occurred. Some of the WHO-led researchers are veterinarians and zoologists -- they know what animals smell like, and could smell their lingering presence a year later, but were told essentially that it was impossible that they were smelling animals because **there "were no live animals there**".) The researchers noted a mixed smell of animals and disinfectant in some areas of the market, but they were told by the market's manager that they were probably smelling the lingering

stench of rotten meat and sewage, according to a joint WHO-China report. [should we add "according to Laing Wannian etc here? Liang Wannian was the leader of the Chinese research team collaborating jointly with the WHO-led research team. The source for the above description are in the annexes to the official joint WHO-China report released at the end of March 2021.]

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Earlier the same day, the international research team visited Wuhan's larger Baishazhou market, where Xiao had regularly surveyed two sellers of live wild animals. Yet the group was told only frozen food, ingredients, and kitchenware were on offer there. Liang Wannian, an epidemiologist who led the Chinese experts collaborating with the WHO-convened team, says his group had no knowledge of Xiao's data either. [if the above information on what the delegation saw all comes from this same source, maybe we should include his name higher up (The source for the description of what the origins researchers saw on Jan. 31, 2021, comes from the official 120-page joint WHO-China report and its 193-page annexes, not from Liang. Since we are essentially accusing China of concealing the information that Xiao documented, we asked Liang at a press conference in late July 2021 when the Chinese team first knew about Xiao's findings -- that the Huanan market and three others in Wuhan were selling live animals permissive to SARS-CoV-2 infection -- and what research China has done subsequently as a follow-up on this information? Liang gave a very long-winded response in Chinese in which he said

essentially "we didn't have that information in January-February 2021 when the research team was in Wuhan". I think it's important we keep this to demonstrate that we have tried to ascertain what China knew/has done and have given the Chinese researchers the opportunity to respond. In addition, I have emailed China CDC Director George Gao at least twice and not received any response.]

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Among the earliest clusters of infections recorded in Wuhan, one involved three Covid cases among staff working at a stall in Huanan. One of the employees, a 32-year-old who fell ill on Dec. 19, traded goods back and forth between the Huanan and Baishazhou markets.
WHERE DOES THIS FACT COME FROM? NOT THE REPORT, RIGHT? WHEN DID IT FIRST BECOME KNOWN? This information was from the joint WHO-China report released in March 2021, however, the WHO-led researchers went to the Baishazhou market more as a demonstration by the China team of what a perfectly functioning large food market looks like. The WHO-led team was oblivious to Xiao's research that showed there were at least two stalls in Baishazhou that had been selling live wild animals for human consuption. So this detail and its significance was lost on the WHO researchers at the time.)

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A confirmed case linking two markets that sold wild animals is "very intriguing," says Stephen Goldstein, a research associate in evolutionary virology at the University of Utah in Salt Lake City. But tracing any contact the

employee might have had with infected wildlife is impossible now that the animals are long gone.WOULD TIMELY PUBLICATION OF THE REPORT HAVE CHANGED THIS? It's unlikely that Xiao et al's paper would have been *published* soon after It was drafted in February 2020, but it could have been *released* as a pre-print ahead of publication and peer-review that same month. That would have **confirmed** what almost everyone had suspected: that there **was** a flourishing wildlife trade in Wuhan that provides a plausible pathway by which coronavirus-infected wildlife from Yunnan and beyond could have introduced the virus to the city, sparking the Covid outbreak. It's also possible that swift recognition of these potential wild-animal vectors could have allowed scientists to test them for the virus and for antibodies against the virus while they were still alive (perhaps not the ones from Huanan, but wildlife in the three other Wuhan markets). Because of the statements the Chinese authorities had been making in January and February that strongly supported the animal spill over theory, it was assumed that this research was being done. Of course, the pandemic was raging then, so it would have been challenging. But, because of the denial by Chinese researchers that wild animals were being sold in Wuhan wet markets before the pandemic, there was never that level of follow up. At least, if there was, it was never made public.)

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"It seems to me, at a minimum, that local or regional authorities kept that informationWHAT INFORMATION? (that Wuhan had a

flourishing live wild animal business going on in its wet markets before the pandemic) quiet deliberately," Goldstein says. "It's incredible to me that people theorize about one type of cover-up, but an obvious cover-up is staring them right in the face."

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From: Peter Daszak [b6]
Sent: 8/17/2021 12:37:54 PM
To: Morens, David (NIH/NIAID) [E] [b6]; Jason Gale [j.gale@bloomberg.net]
CC: b6; Garry, Robert F [b6]; b6; b6
Subject: RE: feedback on wild animals

Importance: High

Very much enjoying your piece out today Jason!

https://www.bloomberg.com/news/features/2021-08-17/where-did-covid-come-from-report-on-infected-wuhan-wild-animals-sheds-new-light

Delayed Wuhan Report Adds Crucial Detail to Covid Origin Puzzle

A study documenting the trade in live wild animals at Wuhan wet markets stayed unpublished for more than a year.

Wuhan Huanan Wholesale Seafood Market, December 31, 2019.

Photo illustration by 731; Photo: Oriental Images

By

Jason Gale

August 17, 2021, 12:01 AM EDT

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The origin story of Covid-19 remains a mystery mired in contentious geopolitical debate. But a research paper that languished in publishing limbo for a year and a half contains meticulously collected data and photographic evidence supporting scientists' initial hypothesis—that the outbreak stemmed from infected wild animals—which prevailed until speculation that SARS-CoV-2 escaped from a nearby lab gained traction.

According to the report, which was published in June in the online journal Scientific Reports, minks, civets, raccoon dogs, and other mammals known to harbor coronaviruses were sold in plain sight for years in shops across the city, including the now infamous Huanan wet

market, to which many of the earliest Covid cases were traced. The data in the report was collected over 30 months by Xiao Xiao, a virologist whose roles straddled epidemiology and animal research at the government-funded Key Laboratory of Southwest China Wildlife Resources Conservation and at Hubei University of Traditional Chinese Medicine.

In May 2017, Xiao began surveying 17 shops at four Wuhan markets selling live wild animals. He was trying to find the source of a tick-borne, Lyme-like disease that had spread in Hubei province years earlier. He kept up monthly visits until November 2019, when the discovery of mysterious pneumonia cases that heralded the start of the Covid pandemic brought his visits to an abrupt end. As the virus started to explode, Xiao recognized the potential significance of his data. In January of 2020, he collaborated with Zhou Zhaomin, a researcher at a wildlife resources laboratory affiliated with China's Ministry of Education, and three seasoned scientists from the University of Oxford's Wildlife Conservation Research Unit, on a manuscript that was submitted to a journal the following month. (They declined to name the publication). "We'd imagined that the journal we sent it to would say, 'Fantastic! Of course we want these data out as quickly as we can. The World Health Organization would be absolutely thrilled to receive this information,'" says Chris Newman, a British ecologist who is one of the paper's co-authors. But it was rejected. "They did not think it would have widespread appeal," says Newman.

Had the study been made public right away, the search for the origins of the virus might have taken a very different course. Not only did the study contain conclusive evidence that live animals were being sold for human consumption at the epicenter of the outbreak, but Newman says he assumes Xiao collected blood-sucking ticks from the wild animals he studiously cataloged. The blood meals of frozen tick samples could be examined for traces of the coronavirus, which would be extremely helpful in identifying infected species prior to December 2019. Xiao didn't respond to emails requesting comment.

In the first months of the epidemic, local researchers asserted that the new coronavirus resembled a spillover from animals, reminiscent of the emergence of the virus that caused severe acute respiratory syndrome (SARS) in wet markets in Guangdong almost 20 years ago. They also readily acknowledged the presence of "a variety of live wild animals" at Wuhan markets.

Raccoon dog, Wuhan wet market, 2013.

Photo illustration by 731; Photo: Animal Equality

The Huanan market was shuttered in the early hours of Jan. 1, 2020, and its 678 stalls emptied and sanitized. In the middle of the month CNN broadcast unverified footage reportedly recorded in early December showing caged deer, marmots, and raccoon dogs there. Photographs of a menu board advertising the price and availability of exotic animals circulated online.

Disease detectives arriving from Beijing on the first day of 2020 ordered environmental samples to be collected from drains and other surfaces at the market. Some 585 specimens were tested, of which 33 turned out to be positive for SARS-CoV-2. "All current evidence points to wild animals sold illegally," China Center for Disease Control Director George Gao and colleagues wrote in the agency's weekly bulletin in late January. All but two of the positive specimens came from a cavernous and poorly-ventilated section of the market's western wing, where many shops sold animals.

"We have found out which stalls on the seafood market in Wuhan had the virus," Tan Wenjie, a researcher at China CDC's viral disease control and prevention institute, was quoted telling the state-owned China Daily newspaper days later. "It is an important discovery, and we will investigate which animal was the source."

How Researchers Missed Clues in Wuhan

How Researchers Missed Clues in Wuhan

China temporarily banned the wildlife trade. The decision became permanent a month later and widened to prohibit human consumption of terrestrial wild animals.

A WHO-China joint mission to Wuhan to examine China's response to the outbreak in February 2020 reported that an effort was under way to collect detailed records on the source and type of wildlife species sold at the Huanan market and the destination of those animals after the market was closed. But there's no public record of that ever happening.

"Unfortunately, the apparent lack of direct animal sampling in the market may mean that it will be difficult, perhaps even impossible, to accurately identify any animal reservoir at this location," Zhang Yongzhen and Edward Holmes, the scientists who published the first genetic sequence of SARS-CoV-2, wrote in a commentary published in the journal Cell in March 2020.

As other nations began blaming the Chinese Communist Party for the pandemic, the government grew defensive. It may have been embarrassed that its citizens were still eating wild animals bought in wet markets—a well-known path for zoonotic disease transmission that China tried unsuccessfully to outlaw almost 20 years ago.

Australia in April 2020 called for a global inquiry into the origins of the pandemic, including China's handling of the initial outbreak. Days later, then-U.S. Secretary of State Mike Pompeo used part of his Earth Day message to call on China to close its wet markets to "reduce risks to human health inside and outside of China."

In response, Geng Shuang, a spokesman for China's Foreign Ministry, denied "wildlife wet markets" existed in the country. Government researchers now dismiss the market hypothesis completely. "SARS-CoV-2 could not have possibly evolved in an animal market in a big city and even less likely in a laboratory," said a paper released in July, written by 22 researchers from mostly government-funded laboratories attached to the Chinese Academy of Sciences.

A more recent paper by government-affiliated scientists contends that the virus may have been imported from multiple locations worldwide, including parts of Europe where mink are raised in areas inhabited also by horseshoe bats known to harbor coronaviruses. "The official narrative changed not because the evidence changed," says Robert Garry, a professor of microbiology and immunology at Tulane University's School of Medicine in New Orleans. "A spillover from a wet market was what caused SARS, and, embarrassingly for China, those wet markets were never shut down." Garry is the co-author of one of the earliest papers on the origins of Covid but wasn't involved in the research on Wuhan's markets.

Since he was not connected to a law enforcement agency, Xiao was granted "unique and complete access to trading practices," he and his colleagues wrote. Seven of the shops he surveyed were in the Huanan market, which has been linked to two of the earliest documented cases of Covid-19. On each visit, Xiao asked vendors what species they had sold over the preceding month, documenting both their numbers and prices.

Xiao checked the animals for injuries and disease, noting that almost a third bore trapping and shooting wounds consistent with being caught in the wild, and that none of the shops displayed an origin or quarantine certificate, making the commerce "fundamentally illegal," according to the study.

His animal logs included masked palm civets and raccoon dogs—both involved in the 2003 SARS outbreak—and other species susceptible to coronavirus infections, such as bamboo rats, minks, and hog badgers. Of the 38 species Xiao documented, 31 were protected.

A closed seafood wholesale market in Wuhan on Jan. 23, 2021.

Photo illustration by 731; Photo: AP Photo

Anyone caught violating China's wild animal conservation law faces fines and up to 15 yea imprisonment. But enforcement was lax, as evidenced by the fact that many of the Wuhan sh displayed their wares openly, "caged, stacked and in poor condition," Xiao observed in the r rt. Xiao estimated that 47,381 wild animals were sold in Wuhan over the survey period luxury food items priced at up to $25 a kilogram ($11 per pound)—or more than fou than pork, China's main meat staple.

The initial manuscript was revised twice following feedback by a reviewer, and after months of exchanges, was rejected.

The researchers revised the manuscript a third time and included data on China's networks (an earlier study, later contested, had implicated pangolins in the virus's sp o humans). In October 2020 they sent it to Scientific Reports.

Springer Nature, the publisher of Scientific Reports, forwarded a copy swiftly to th of an agreement with the agency, says Ed Gerstner, Springer Nature's director of journals, policy, and strategy. But the publisher emailed the paper, titled "Pangolin Trading in China: Wuhan's Alibi in the Origin of Covid-19," to a generic address at the WHO that functions as an inbox for unpublished research where it languished amid tens of thousands of submissions flooding the agency.

Springer Nature also sent a copy to Maria Van Kerkhove, the organization's technical lead for Covid-19. Van Kerkhove says there were so many submissions related to the pandemic that she didn't look at it right away, and she regrets there was no direct follow-up from the journal or by the authors. "It's a shame this important information was not shared directly with the mission team while the team was in Wuhan and visited the markets," she said in an email. "This paper would certainly have added great value."

Newman says his Chinese co-authors never told him why they didn't take their data directly to the WHO, but it's possible they were more comfortable writing a report on market surveys for publishing in a journal, he says.

The China-based researchers would have had reason to be cautious. In February 2020, the China CDC prohibited scientists working on Covid-related research from sharing their data and required them to receive permission before conducting any studies or publishing the results. Days later, a special panel convened by China's top executive body to oversee coronavirus research took control of all publication work related to the pandemic for "coordinated deployment."
An international group of experts convened by the WHO to research the origins of Covid traveled to Wuhan earlier this year—a trip that might have yielded different results if the scientists had known about Xiao's work.

By the time the team visited the Huanan market in the afternoon of Jan. 31—more than a year after its closure—little remained to assist the kind of epidemiological sleuthing that led SARS investigators to Himalayan palm civets, raccoon dogs, and Chinese ferret-badgers sold in live-animal markets in Guangdong almost two decades ago.
The researchers noted a mixed smell of animals and disinfectant in some areas of the market, but they were told by the market's manager that they were probably smelling the lingering stench of rotten meat and sewage, according to the official joint WHO-China report released in March 2021. Chinese officials briefing the visitors told them 10 Huanan shops had been found to be selling frozen "domesticated" wild animals, including bamboo rats—some sourced from Yunnan province, where scientists found a coronavirus that most closely matches SARS-CoV-2 in horseshoe bats. But no live animals had been seen before the market was closed, the official said.
The researchers saw nothing to dispute that. They were invited to quiz two Wuhan residents whom they were told had shopped there regularly for 20 and 30 years and who, according to the report, said they "had never witnessed any live animals being sold."

Earlier the same day, the international research team visited Wuhan's larger Baishazhou market, where Xiao had regularly surveyed two sellers of live wild animals. Yet when the researchers were there they were told that only frozen food, ingredients, and kitchenware were on offer. Liang Wannian, an epidemiologist who led the Chinese experts collaborating with the WHO-convened team, says his group had no knowledge of Xiao's data either.
Among the earliest clusters of infections recorded in Wuhan, one involved three Covid cases among staff working at a stall in Huanan. One of the employees, a 32-year-old who fell ill on Dec. 19, traded goods back and forth between the Huanan and Baishazhou markets.

A confirmed case linking two markets that sold wild animals is "very intriguing," says Stephen Goldstein, a research associate in evolutionary virology at the University of Utah in Salt Lake City. But tracing any contact the employee might have had with infected wildlife is impossible now that the animals are long gone. As for the existence of a flourishing live wild animal business, "It seems to me, at a minimum, that local or regional authorities kept that information quiet deliberately," he says. "It's incredible to me that people theorize about one type of cover-up, but an obvious cover-up is staring them right in the face."
U.S. intelligence agencies will report their own findings on Covid's origins later this month. But with only circumstantial evidence remaining, the world may now never know what caused the outbreak. "It is unclear why earlier initiatives within China to locate source animals for SARS-CoV-2 were curtailed, and now appear unfortunately to have stopped," says Tulane's Garry. "Instead, the focus is on highly implausible origin scenarios. If we continue to place politics over science, humanity will again be unprepared for the next emergence of a pandemic virus."

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Morens, David (NIH/NIAID) [E] b6
Sent: Sunday, August 15, 2021 6:26 PM
To: Jason Gale <j.gale@bloomberg.net>
Cc: b6; Garry, Robert F
b6
Subject: RE: feedback on wild animals

Jason, not sure whose office politics is worse, but there is many a day I'd rather be in Australia, even if I had to drink that awful beer Fosters. (To quote Grouco Marx as he help up a glass of questionable beer: "last time I saw something like this, they had to shoot the horse")…. But I

do have fond memories of Australia, and b6, are out in b6, the only large city there (if oy can call it that) I have never been, although it's high on my bucket list if this damn pandemic ever ends. b6

b6

David

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520

☎ b6 (assistant: Whitney Robinson)
301 496 4409
b6

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 7:54 PM
To: Morens, David (NIH/NIAID) [E] b6
Cc: b6; Garry, Robert F b6
Subject: Re: feedback on wild animals

Thanks, David.
I just got off a video conf call with the current editor in NY (the one asking all the latest questions). He's very nice and I feel like he gets its. He thinks it's a fascinating story.
Problem we have in journalism is that there are some people who aren't interested in actual journalism and telling stories; they want to climb to the top and manage people. I [b6], but am so grateful that I still get to meet so many remarkable people, visit cool places and see all aspects of humanity.
A difficulty working for a NY-based organization is that it's assumed that all the best people are in NYC, so being in Melbourne ([b6] [b6]), is that folks consider Australia a backwater and, by association, I mustn't be all that important/valuable. But my new role as "global biosecurity czar" is helping somewhat.
Ahhhhh... Office politics!

From: [b6] At: 08/15/21 09:03:03 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: [b6], [b6]
Subject: Re: feedback on wild animals

Jason, all I can say is that it must be harder to be a journalist than a scientist…. I had no idea someone at your level would get pushed back.

Usually i get from one to 4 reviewer responses to a ms. and three of those are out to lunch. Occasionally i get a reviewer who really understands the work: half of those are helpful, the other half are trashers.

Maybe it's like being in the government where i am: there is endless push back, but the push-backers are brainless idiots who don't know what they are talking about.

Do you ever get to a point where the editors leave you alone on the science? Or do they all think they are science geniuses? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 14, 2021, at 16:05, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Thanks, Bob. In journalism, getting to the truth even when it's genuinely and actively pursued, can be a tortuous process!

----- Original Message -----
From: Robert F Garry [b6]
To: JASON GALE, [b6], [b6],

b6
At: 08/15/21 01:45:11 UTC+10:00

Looking forward to this important article. Truth is stronger than fiction.

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 6:41 AM
To: b6 ;
b6 ;
Garry, Robert F b6
b6
Subject: Fwd:Re:feedback on wild animals

External Sender. Be aware of links, attachments and requests.

Hi guys, in case you have nothing better to read over the weekend, this is some of the dialog I am having with editors in the U.S. Essentially, my response to questions from editor #6.
I thought I could claim victory when it looked like the story could be published this morning, but b6 of Businessweek objected and thinks b6 can make it better with another revision and an ETA of Tuesday.
Sigh.
Jason

From: Jason Gale (BLOOMBERG/ NEWSROOM:) At: 08/14/21 16:36:58 UTC+10:00
To: Cristina Lindblad (BLOOMBERG/ NEWSROOM:) , Eric Gelman (BLOOMBERG/ NEWSROOM:)
Cc: Joel Weber (BLOOMBERG/ NEWSROOM:)
Subject: Re:feedback on wild animals

> Hi.
> Thanks for the feedback and the questions, which I have tried to answer in detail.
> It's often easier to have a conversation over Nexi or the phone to explain nuanced information, or details that are clear to me, but might not be to someone coming at this fresh, but that's tricky with the time difference.

When you look back at what's happened here, it seems that in trying to deflect blame for the pandemic, which became increasingly vicious as the cataclysmic nature of the Covid-19 pandemic unfolded around March-April 2020, China tried to conceal a very obvious, very plausible source of the pandemic: it's flourishing wildlife trade (worth about $90 billion in 2016).
It was an obvious cause of the pandemic because an almost identical scenario triggered an international outbreak caused by a very similar coronavirus (SARS) in 2003-04. But in attempting to cover up the wildlife trade, and making like there were never any wild animals being sold in Wuhan's wet markets, things began to backfire on China; questions were raised about the nearby lab studying these coronaviruses. The more geopolitical, heated and vicious the arguments and accusations became, the less cooperative China became. In response, the more intent/adamant some groups have become in their belief that China is covering up a lab-leak. It's become a vicious circle. If China isn't coming clean on the wild animals, what else is it trying to hide??
China's defensiveness means we may never get the cooperation needed to find the answers. If China had been honest and transparent about the wild animals in the wet markets, it might not be in this mess now.

Anyway, here are my response to your queries in green.

By the way, I wonder if the current headline:
Delayed Paper Gives Credence to Wuhan Market Covid Origin Story

doesn't convey much more than what we had 2 months earlier when we reported Xiao's findings: China Markets Sold Mink, Civets, Stoking Natural Origins Theory
Perhaps Obscured China Paper Scuppered Chance to Trace Covid Origins
would hits "China", "Covid" and "Origins" in a way that won't alienate people who already believe it was a lab-leak

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According to the report, minks, civets, raccoon dogs and other mammals known to harbor coronaviruses were sold in plain sight for years in shops across the city, including the now infamous Huanan wet market, to which many of the earliest Covid cases were traced. The evidence collected over 30 months by **Xiao Xiao** a I'D NAME HIM HERE [[i don't know that naming a researcher no one has ever heard of adds much]] researcher working at a lab affiliated with China's Ministry of Education was hastily drafted into a manuscript and submitted to a scientific journal [Joel, we cannot name the first journal they delivered it to because the authors decline to give us the name, saying it may affect their future chances of being published] in February 2020, just weeks before the outbreak was declared a pandemic.

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While the study received wide attention when it was eventually released by a different publisher [Publisher (Springer Nature) and publication (Scientific Reports) are different. Might add confusion DO YOU WANT TO ADD NAME HERE?], its long and torturous journey to

publication gave **Chinese officials an opportunity to weave alternative narratives in which the virus may have come from abroad, even from a U.S. Army biological research facility.** [the stuff in bold is not contested and we go on to show how that happened, documenting with links when available]I TWEAKED WORDING HERE BUT THIS STILL ISN'T QUITE RIGHT. IF THE REPORT HAD BEEN PUBLISHED EARLIER CHINESE OFFICIALS COULD STILL HAVE DONE WHAT THEY DID—THEY COULD HAVE JUST SAID THE REPORT WAS SHODDY, OR WHATEVER, AND THEY ALSO COULD HAVE JUST IGNORED IT. I disagree. The evidence that Xiao et al provide was meticulously documented and supported by photographs that would have been difficult/impossible for China to dismiss (as older published photos and media reports had been). What's more, Newman understands that Xiao collected blood-sucking ticks from the wild animals he studiously cataloged. His frozen tick samples could be tested for blood/antibodies/virus, which could be extremely helpful in identifying infected species PRIOR to December 2019. The WHO team knew nothing of this, so couldn't have asked China for this research or any results, had they actually done the research. The delay in the publication of Xiao's paper delayed the evidence that there were live animals sold in the Huanan market. The WHO researchers couldn't have asked about tests on wildlife that ostensibly were never there. Likewise, Chinese authorities couldn't have done the tests on animals that didn't exist. The problem is that in January and February 2020, it was widely assumed the animals HAD been there and that the necessary tests and tracing of animals (the

sampling of animals on farms they were raised on, testing of farm workers, animal hunters, transporters and traders **had all been done** by researchers in China -- the very things that ultimately led to the discovery of the origins of SARS and of MERS viruses. **None of these things were done** (or at least, there is nothing publicly available to show that they were done) **because the animals "weren't there"**. Now that we all know they were there, China as lost considerable face. The issue has become so political than there is much less/?no willingness to cooperate and conduct the additional research that the WHO-led team recommended. Ideally, Xiao's ticks should be studied, but it's doubtful that will now happen (I wouldn't be surprised if he's been ordered to incinerate them!) Hope this is clear.

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An international team of experts convened by the WHO traveled to Wuhan earlier this year to seek answers—a trip that might have yielded different results if the scientists had known about the work of Xiao Xiao, a virologist whose roles straddled epidemiology and animal research at the government-funded Key Laboratory of Southwest China Wildlife Resources Conservation and at Hubei University of Traditional Chinese Medicine.‘MIGHT HAVE YIELDED DIFFERENT RESULTS’ SEEMS HIGHLY SPECULATIVE. WHAT RESULTS DID IT YIELD? I’D CUT THIS GRAF As mentioned above, the WHO-convened team of researchers was told by market authorities, vendors and regular market visitors that there were no live animals sold in the Huanan market. That undermined completely the

premise that the Huanan market was the kind of place where live animals from different species are stacked in cages, with urine and fecal material drips from one cage to the one below it, where there are splatters of blood and guts from animals, and lots of potential for the spread of diseases from one species to another (including Homo sapiens). Instead, the market (and one other one known to have been selling live wild animals in 2019) was presented as selling only frozen wild animals and aquatic species and things unlikely to be the source of SARS-CoV-2. The WHO researchers were told there were frozen ferret badgers and other wildlife found in freezers. Some of the carcasses actually came from Yunnan, the province where the closest coronavirus related to SARS-CoV-2 was found in bats -- thus establishing a potential route from Yunnan to Wuhan in wildlife. That was actually important. When the researchers showed their Chinese counterparts photos of caged raccoon dogs taken in the Huanan market by Prof. Edward Holmes five or six years earlier, they were told by Chinese scientists that the photos may have been faked, and that the market had ceased selling such live animals anyway. The WHO researchers saw no evidence (empty cages, animal pelts, etc) to dispute what the Chinese scientists told them, although they did smell "animals" -- but were told they were smelling rotten meat, sewage etc. Three people associated with the WHO-led mission told me that they didn't believe the information they were given by anyone associated with the market. But since there was no evidence to the contrary, they were unable to push the Chinese

scientists further on this. **The fact that the mission concluded that frozen, not live wild animals were sold in the market undermined the thesis that Covid resulted from an animal spillover.** And the absence of strong evidence pointing to a spill over from wild animals to humans made the lab-leak theory look, in relative terms, more plausible.

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Six months and two revisions later, the journal's publishers rejected the paper. "They did not think it would have widespread appeal," says Newman, who declined to name the publication [do you want us to say why he won't name it? I don't think it's necessary. There's no upside for scientists to make a publisher look bad (most journals are published by a handful of publishers)] "It caused us, especially our Chinese co-authors, concern that these data would not be taken seriously."

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The manuscript underwent a third revision to include data on China's pangolin trade networks (an earlier study, later contested, had implicated pangolins in the virus's spread to humans)WHO ASKED THAT THIS INFO BE INCLUDED? (I did, because we're trying to explain here why WHO missed the significance of Xiao's evidence. The WHO was slammed by other papers being submitted PLUS Xiao's paper had a weird title. That title seemed relevant to the authors back in January-February 2020, when pangolins were considered a possible SARS-CoV-2-spreading culprit. But in October 2020, pangolins were off the hook, so the title would have seem

irrelevant/unimportant at first glance at WHO). It was then sent to the online journal *Scientific Reports*.

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The China-based researchers had reason to be cautious. In February 2020, the China Center for Disease Control (CCDC lets not use "CCDC". It's not commonly used like the U.S. "CDC" is, and it's going to force readers to go back and figure out what the "CCDC" is) prohibited scientists working on Covid-relate

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~~CCDC~~ Disease detectives arriving from Beijing (think this makes it clear that these are China's disease "feds" arriving) on the first day of 2020 ordered environmental samples to be collected from drains and other surfaces at the market. Some 585 specimens were tested, of which 33 turned out to be positive for SARS-CoV-2. "All current evidence points to wild animals sold illegally," China CDC Director George Gao and colleagues wrote in the agency's weekly bulletin in late January. All but two of the positive specimens came from a cavernous and poorly-ventilated section of the market's western wing, where many shops sold animals.

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~~The information void kindled a raging political debate that's already caused a trade war between China and Australia, as nations demand to know how Covid emerged.~~ Australia in April 2020 called for a global inquiry into the origins of the pandemic, including China's

handling of the initial outbreak. Days later, then-U.S. Secretary of State Mike Pompeo used part of his Earth Day message to call on China to close its wet markets to "reduce risks to human health inside and outside of China."

Based on a discussion today with someone with knowledge of the WHO-led mission to Wuhan in Jan-Feb 2021, a significant catalyst for China's defensiveness is the emergence of **claims for reparations** that extended from the finger-pointing at China's wet markets. These surfaced in April 2020, and have continued as recently as June 2021 (when Trump pushed for it again at a rally in the Midwest)

SO IS THIS THE POINT AT WHICH CHINA, WHICH SEEMINGLY ACCEPTED THE WET MARKET HYPOTHESIS, BEGAN TRYING TO CREATE A DIFFERENT NARRATIVE? (I think it was incremental. I believe China was embarrassed that its citizens were still buying wild animals in wet markets to eat -- a well-known hazard for zoonotic disease transmission that China tried unsuccessfully to outlaw almost 20 years ago. But that embarrassment/humiliation morphed into rigid denial and obfuscation when governments began openly blaming the Chinese Communist Party and agitating for China to pay reparations for the pandemic. See these clips:

- USA Today: Blame the Chinese Communist Party for the coronavirus crisis: Coronavirus crisis proves communism is still a grave threat to the entire world. If Beijing had just been honest, the pandemic could be preventable. April 5, 2020

- Yahoo News: More than half of Americans think China should pay coronavirus reparations, poll shows April 9, 2020
- Voice of America: Americans Join Coronavirus Lawsuit to Make China Pay April 10, 2020
- Washington Post Opinion: China must pay reparations to Africa for its coronavirus failures April 16, 2020
- Reuters: In a first, Missouri sues China over coronavirus economic losses April 22, 2020
- Washington Post: Missouri is suing China over the coronavirus pandemic. It's the latest conservative gambit, April 22, 2020
- New York Post: Top German paper demands $165 billion coronavirus reparations from China April 22, 2020
- Attorney General Fitch Prepares to Sue China on Behalf of Mississippians April 22, 2020
- Newsweek: Trump on U.S. Seeking Compensation From China Over COVID-19: 'We Have Not Determined the Final Amount' April 28, 2020
- The Guardian: Trump says China could have stopped Covid-19 and suggests US will seek damages April 28, 2020
- Intelligencer: Trump Thinks He Can Make China Pay for the Virus Like Mexico Paid for the Wall, April 30, 2020
- Washington Post: U.S. officials crafting retaliatory actions against China over coronavirus as President Trump fumes April 30, 2020
- Lawfare: Does China Really Owe the World Trillions of Dollars? May 7, 2020
- Fortune: Trump's demand that China pay coronavirus

reparations evokes an ugly history May 8, 2020

- South China Morning Post: Why China won't be paying the West coronavirus reparations any time soon May 15, 2020
- Deccan Herald, India: Abhijit Bhattacharyya | Why China needs to pay reparations to the world June 4, 2020
- Why calls for reparations from China for coronavirus are an unfeasible distraction June 9, 2020
- Newsweek: Trump Demands China 'Pay Reparations' for COVID, Says $10 Trillion Not Enough June 12, 2021

In response, Geng Shuang, a spokesman for China's Foreign Ministry, denied "wildlife wet markets" existed in the country. Government researchersCAN WE BE MORE SPECIFIC? Twenty-two researchers from mostly nationally-funded laboratories (I think it's the Chinese equivalent of the NIH in the U.S.) and institutes attached to the Chinese Academy of Sciences) now dismiss the market hypothesis completely. "SARS-CoV-2 could not have possibly evolved in an animal market in a big city and even less likely in a laboratory," they wrote in a paper released last month ahead of publicationWHERE/WHEN WILL IT BE PUBLISHED? It was released as a "pre-print" on a Chinese academic repository ahead of publication that appears to be managed/owned by the Chinese Academy of Sciences. Papers are usually released in pre-print form before they have been accepted for publication or peer-reviewed as a way of expediting public access to the information. In this case, there is no information to suggest

if, where or when the paper will be published.

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A more recent paperBY WHOM? (China CDC's Gao and eight other scientists mostly from the Chinese of Academy of Sciences' institutes) contends that the virus may have been imported from multiple locations worldwide, including parts of Europe where mink are raised in areas inhabited also by horseshoe bats known to harbor coronaviruses. "The official narrative changed not because the evidence changed," says Robert Garry, a professor of microbiology and immunology at Tulane University's School of Medicine in New Orleans "A spillover from a wet market was what caused SARS, and, embarrassingly for China, those wet markets were never shut down." Garry is the co-author of one of the earliest papers on the origins of Covid but wasn't involved in the research on Wuhan's markets.

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I DON'T REALLY UNDERSTAND WHAT POINT THIS GRAF IS TRYING TO MAKE. This is intended to demonstrate the kind of gaslighting that has occurred. Some of the WHO-led researchers are veterinarians and zoologists -- they know what animals smell like, and could smell their lingering presence a year later, but were told essentially that it was impossible that they were smelling animals because **there "were no live animals there**".) The researchers noted a mixed smell of animals and disinfectant in some areas of the market, but they were told by the market's manager that they were probably smelling the lingering

stench of rotten meat and sewage, according to a joint WHO-China report. [should we add "according to Laing Wannian etc here? Liang Wannian was the leader of the Chinese research team collaborating jointly with the WHO-led research team. The source for the above description are in the annexes to the official joint WHO-China report released at the end of March 2021.]

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Earlier the same day, the international research team visited Wuhan's larger Baishazhou market, where Xiao had regularly surveyed two sellers of live wild animals. Yet the group was told only frozen food, ingredients, and kitchenware were on offer there. Liang Wannian, an epidemiologist who led the Chinese experts collaborating with the WHO-convened team, says his group had no knowledge of Xiao's data either. [if the above information on what the delegation saw all comes from this same source, maybe we should include his name higher up (The source for the description of what the origins researchers saw on Jan. 31, 2021, comes from the official 120-page joint WHO-China report and its 193-page annexes, not from Liang. Since we are essentially accusing China of concealing the information that Xiao documented, we asked Liang at a press conference in late July 2021 when the Chinese team first knew about Xiao's findings -- that the Huanan market and three others in Wuhan were selling live animals permissive to SARS-CoV-2 infection -- and what research China has done subsequently as a follow-up on this information? Liang gave a very long-winded response in Chinese in which he said

essentially "we didn't have that information in January-February 2021 when the research team was in Wuhan". I think it's important we keep this to demonstrate that we have tried to ascertain what China knew/has done and have given the Chinese researchers the opportunity to respond. In addition, I have emailed China CDC Director George Gao at least twice and not received any response.]

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Among the earliest clusters of infections recorded in Wuhan, one involved three Covid cases among staff working at a stall in Huanan. One of the employees, a 32-year-old who fell ill on Dec. 19, traded goods back and forth between the Huanan and Baishazhou markets.
WHERE DOES THIS FACT COME FROM? NOT THE REPORT, RIGHT? WHEN DID IT FIRST BECOME KNOWN? This information was from the joint WHO-China report released in March 2021, however, the WHO-led researchers went to the Baishazhou market more as a demonstration by the China team of what a perfectly functioning large food market looks like. The WHO-led team was oblivious to Xiao's research that showed there were at least two stalls in Baishazhou that had been selling live wild animals for human consuption. So this detail and its significance was lost on the WHO researchers at the time.)

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A confirmed case linking two markets that sold wild animals is "very intriguing," says Stephen Goldstein, a research associate in evolutionary virology at the University of Utah in Salt Lake City. But tracing any contact the

employee might have had with infected wildlife is impossible now that the animals are long gone.WOULD TIMELY PUBLICATION OF THE REPORT HAVE CHANGED THIS? It's unlikely that Xiao et al's paper would have been *published* soon after It was drafted in February 2020, but it could have been *released* as a pre-print ahead of publication and peer-review that same month. That would have **confirmed** what almost everyone had suspected: that there **was** a flourishing wildlife trade in Wuhan that provides a plausible pathway by which coronavirus-infected wildlife from Yunnan and beyond could have introduced the virus to the city, sparking the Covid outbreak. It's also possible that swift recognition of these potential wild-animal vectors could have allowed scientists to test them for the virus and for antibodies against the virus while they were still alive (perhaps not the ones from Huanan, but wildlife in the three other Wuhan markets). Because of the statements the Chinese authorities had been making in January and February that strongly supported the animal spill over theory, it was assumed that this research was being done. Of course, the pandemic was raging then, so it would have been challenging. But, because of the denial by Chinese researchers that wild animals were being sold in Wuhan wet markets before the pandemic, there was never that level of follow up. At least, if there was, it was never made public.)

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"It seems to me, at a minimum, that local or regional authorities kept that informationWHAT INFORMATION? (that Wuhan had a

flourishing live wild animal business going on in its wet markets before the pandemic) quiet deliberately," Goldstein says. "It's incredible to me that people theorize about one type of cover-up, but an obvious cover-up is staring them right in the face."

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From: Morens, David (NIH/NIAID) [E] [b6 b6]
Sent: 8/20/2021 11:10:06 PM
To: Edward Holmes [b6]
CC: Garry, Robert F [b6 b6]; Kristian G. Andersen [b6]; Jason Gale [j.gale@bloomberg.net]; b6; Peter Daszak [b6]; b6
BCC: Morens, David (NIH/NIAID) [E] [b6 b6]
Subject: Re:
Attachments: Pangolin-Serology-Nido2021-Poster.pdf

Eddie, thanks so much, I had no idea that some of these conflicting data represented bullshit agendas. What has happened to scientific integrity that scientists would sell their souls over dishonest political agendas? I guess i am too naïve…. I have always believed or at least hoped that scientists had the utmost integrity….

If i may impose on you again, last week the Italian group published, finally, their data on viral sequences dating back to early-mid October 2019 and thereafter from Italy, suggesting, or so the data seem to say, that their sequences are upstream of the earliest Wuhan sequences two months later.

If true, this would suggest an earlier viral origin spread to Europe before being detected in Wuhan. The Italian sequences seemed to suggest that the Wuhan virus was a downstream offshoot?

Perhaps I misunderstand, either that or the authors are nuts? Surely you guys can figure this out? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 20, 2021, at 17:25, Edward Holmes b6 wrote:

It's diabolical nonsense David. Irrespective of what they state in that 'paper', Linfa has found serological evidence for closely related viruses in pangolins dating back several years and the HKU team have similar data (see attachment). Plus the Guangdong pangolins have been my multiple groups in different ways and there is an independent lineage in Guangxi.

The attempt to undermine the pangolin data and the people that generated it one of the shameful examples of anti-science I have ever seen. The reality is that is because the RBD of the Guangdong pangolins is genetically similar to SARS-CoV-2 it becomes an inconvenient data point for those who believe the virus came from a lab in Wuhan hence their attempts to undermine it.

Cheers,

Eddie

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PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute for Infectious Diseases & Biosecurity,
School of Life & Environmental Sciences and School of Medical Sciences,
The University of Sydney | Sydney | NSW | 2006 | Australia
T
E b6 <mailto: b6

On 21 Aug 2021, at 1:03 am, Morens, David (NIH/NIAID) [E]
b6 <mailto: b6 wrote:

Thanks to both you and Kristian. Very heelpful to know what the experts think, because 5o us mere mortals, phylogenetic and sequencing interpretation is a bit inscrutable.

Yes, although I don't know her personally, I know OF Alina Chan based on two papers of hers I came across, one of which was a screed against Eddie's recent review. It seemed biased, cherry-picked, and not the work of a scientist with integrity.

<image004.gif>

David M. Morens, M.D.
CAPT, United States Public Health Service
Senior Advisor to the Director
Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A-03
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520
• b6 (assistant: Whitney Robinson)
• 301 496 4409
• b6 <mailto: b6

<image005.jpg>

From: Garry, Robert F b6 <mailto: b6

Sent: Friday, August 20, 2021 10:38 AM
To: Morens, David (NIH/NIAID) [E]
b6 <mailto: b6 >>; Kristian G. Andersen
b6 <mailto: b6
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
<mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6
Subject: Re:

David,

This from a really super young investigator Alex Crits-Christoph. The authors concluded: “(a) the pangolin covs are actually from mice (b) actually, they were actually cloned artificial constructs, (c) actually, there were other viruses in the samples as well (oh no! who'd have thought), (d) actually, it's all contaminated with dog dna.”

My take: It is garbage and no they [the authors] are not ok - although my supposition is that they are being well compensated for generating this nonsense. Alina Chan [who is a quite dangerous IMO young investigator and is writing a book] is using the very same approach - spouting a lot of pseudoscientific garbage, arguing from "authority." etc., but finding a receptive [and likely wealthy] audience that can put the garbage to work. The whole Dr. Yan/Steve Bannon saga is but one of the examples of this approach.

b

From: "Morens, David (NIH/NIAID) [E]"
b6 <mailto: b6
Date: Friday, August 20, 2021 at 8:56 AM
To: Kristian Andersen b6 <mailto: b6
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>,
b6 <mailto: <mailto: b6 ,
b6 <mailto:
b6 <mailto b6
b6 <mailto: <mailto: b6 ,
b6 <mailto: <mailto: b6 ,
b6 <mailto: <mailto: b6 , Robert Garry
b6 <mailto: <mailto b6
<mailto: b6 ,
b6 <mailto: <mailto b6
Subject: <no subject>

External Sender. Be aware of links, attachments and requests.
Do you all know these data? see link below….
[2108.08163] Cloning vectors and contamination in metagenomic datasets raise concerns over pangolin CoV genome authenticity (arxiv.org)<https://protect-au.mimecast.com/s/s7cRCQnMBZfkxWRNQTxp1lD?domain=nam11.safelinks.protection.outlook.com>

<image006.gif>

David M. Morens, M.D.
CAPT, United States Public Health Service
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• b6 (assistant: Whitney Robinson)
• 301 496 4409
• b6 <mailto: b6

<image007.jpg>

From: Kristian G. Andersen b6 <mailto: b6
Sent: Thursday, August 12, 2021 8:11 PM
To: Morens, David (NIH/NIAID) [E]
b6 <mailto: b6
Cc: Jason Gale <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 ; Garry, Robert F
b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6
Subject: Re: The story behind the missing story about the story behind the missing raccoons

I hear La Jolla has some pretty nice beaches - just saying.

Oh wait, I live here - here's what's outside my office:

<image008.jpg>

Happy to save you a spot - you know, 'field' research.

K

On Thu, Aug 12, 2021 at 5:09 PM Morens, David (NIH/NIAID) [E]
b6 <mailto b6 wrote:

You deserve that beach! Reminds me of that Warren Zevon song about “sippin’ Fosters in the shade”…. Mr. Bad example, i think it was…. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Aug 12, 2021, at 20:00, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>> wrote:

Thanks, David. I've actually been tied up with a podcast series on long Covid (while trying to stay on top of the usual vaccine effectiveness stuff. Busyness with which y'all are only too familiar!). But it helps to vent sometimes about you can feel pretty defeated by your job. Thanks for the support. There will be a beach for me to lay on somewhere some day... JG

From: b6 <mailto: b6 At: 08/13/21 09:05:19 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:) <mailto:j.gale@bloomberg.net> , b6 <mailto: b6 <mailto: b6 b6 mailto: b6 , <mailto: b6 , b6 <mailto: b6 , b6 <mailto: b6 , b6 <mailto: b6 <mailto: b6 b6 <mailto: b6
Subject: RE: The story behind the missing story about the story behind the missing raccoons

Jason, yikes!, but it is a miracle that with all that work you have still been able to crank out multiple high-calibre articles. I have no idea why anyone up your chanin would jerk you around. Who are these guys anyway???? Just keep doing it and overcome, OK?

<image006.gif>

David M. Morens, M.D.
CAPT, United States Public Health Service

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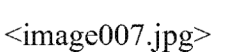
• b6 <mailto: b6



From: Jason Gale (BLOOMBERG/ NEWSROOM:)
<j.gale@bloomberg.net<mailto:j.gale@bloomberg.net>>
Sent: Thursday, August 12, 2021 5:53 PM
To: b6 <mailto: b6 ;
b6 <mailto: b6 ;
b6 <mailto: b6 ; Morens, David
(NIH/NIAID) [E] b6 <mailto: b6 ;
b6 <mailto: b6
b6 <mailto: b6 ;
b6 <mailto: b6 ; Garry, Robert F
b6 <mailto b6 <mailto: b6
b6 <mailto: b6
Subject: The story behind the missing story about the story behind the missing raccoons

Hi everyone,
Just letting you know that my story has been turned into a sh!tshow internally. My long awaited feature on why the raccoon dogs were there in Wuhan one minute, gone the next and why we waited 18 months to find out for sure that they were there in the first place, has taken more twists and turns than any Olympic diver, thanks to some egomaniac editors. (Please keep that bit to yourselves).
I have even more sympathy for Xiao et al. I'm told now Tuesday for publication, but I wouldn't be surprised if some a-hole higher up the food chain spikes it. To say I am exasperated (and a tad emotional after working 13 days straight) is an understatement.
Kindest regards,
Jason

One Health investigation of exposure to SARS-related coronaviruses in trafficked Sunda pangolins (*Manis javanica*)

Brian M. Worthington[1,2,3], Portia Wong[4], Kishoree K. Kumaree[1,2,3], Tracey Prigge[4], Kar Hon Ng[1], Paolo Martelli[5], Shelby McIlroy[4], Yunshi Liao[1], Marcus H.-H. Shum[1], Elliott F. Miot[1,6,7], William Y.-M. Cheung[1,2,3], Helen C. Nash[8], Wirdateti[9], Gono Semiadi[9], Caroline Dingle[4], Oliver G. Pybus[10,11], Edward C. Holmes[12,13], Gabriel M. Leung[1,13], Yi Guan[1,2,3,13], Huachen Zhu[1,2,3,13], Timothy C. Bonebrake[4], Tommy T.-Y. Lam[1,2,3,6,13*]

Introduction

Early in the COVID-19 pandemic, Sunda pangolins (*Manis javanica*) involved in illegal wildlife trade were identified as hosts of SARS-related coronaviruses (SARSr-CoVs). We have investigated 89 Sunda pangolin carcasses seized in 2013 (n=1) and 2018 (n=88) by Hong Kong authorities. We aimed to examine the virome of these animals, to determine any previous exposure to SARSr-CoVs, and to identify the origin of these animals from wild populations throughout Southeast Asia.

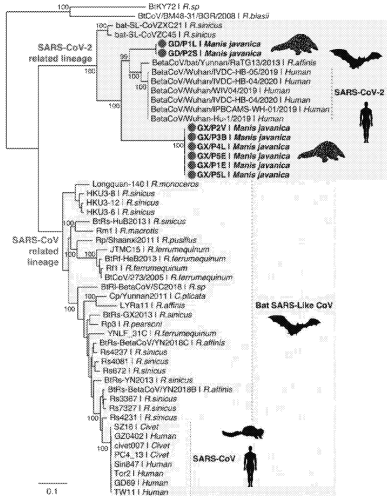


Fig. 2. Sarbecovirus phylogeny with SARSr-CoVs discovered in seized pangolins in China. Figure from Lam et al. 2020. Maximum likelihood tree constructed from concatenated coding regions using GTRGAMMA nucleotide substitution model with 1,000 bootstrap replicates.

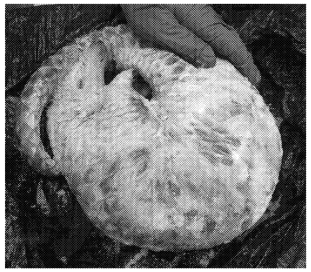


Fig. 1. Pangolin carcass seized by Hong Kong authorities during anti-smuggling operations.

Methods

Post-mortem examination

Collection of swabs, fluids, and tissue samples using microbiological best practices.

Detection of coronaviruses

SARS-CoV-2 specific RT-qPCR (Hybribio) and conventional RT-PCR with universal CoV primers were performed on 504 swab and tissue samples.

SARS-CoV-2 total Ab ELISA

Double-antigen bridging assay (Wantai) used to detect anti-SARS-CoV-2 spike antibodies in 168 blood and other body fluid samples.

Population genomics

Double-digest RAD-Seq performed with downstream SNP calling following Nash et al. (2018). Principle Component Analysis (PCA) conducted using SNPRelate package in R.

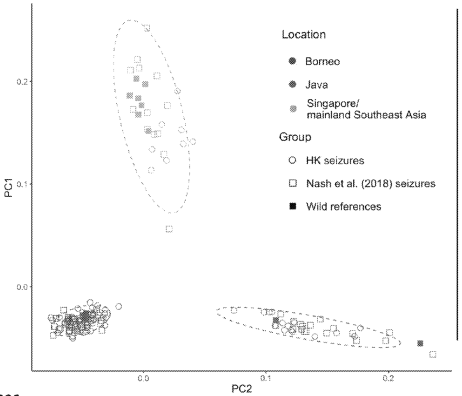


Fig. 3. PCA biplot of individuals in 2018 Hong Kong seizure, seized individuals from Nash et al. (2018), and wild references based on 2,723 SNPs. PC1 (y-axis) accounts for 2.82% of variation, while PC2 (x-axis) accounts for 2.29% of variation. Ellipses represent 95% confidence interval of each cluster.

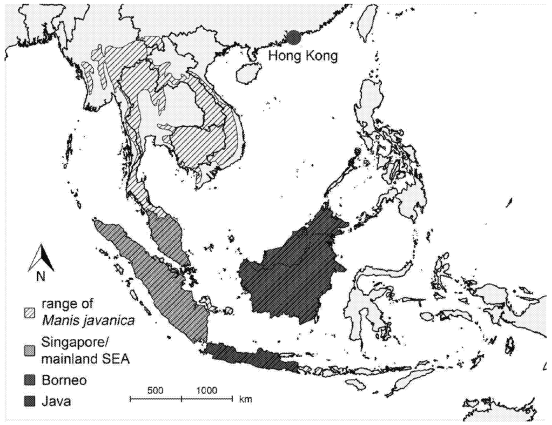


Fig. 4. Range distribution of *Manis javanica* and estimated origins for animals in the 2018 HK seizure. Range data extracted from the IUCN Red List of Threatened Species (2021).

Results

- Six pangolins from the 2018 HK seizure were found to be seropositive and another three were borderline seropositive using a double-antigen bridging assay to detect antibodies cross-reactive with SARS-CoV-2 spike.
- Multiple samples tested seropositive or borderline from four individuals.
- None of the swab or tissue samples screened by PCR tested positive for CoVs.
- Based on PCA results, seropositive individuals were determined to originate from populations in Borneo, Java, and Sumatra or mainland Southeast Asia.
- Consistency in slaughter and dressing method of carcasses may indicate centralized processing of live animals.

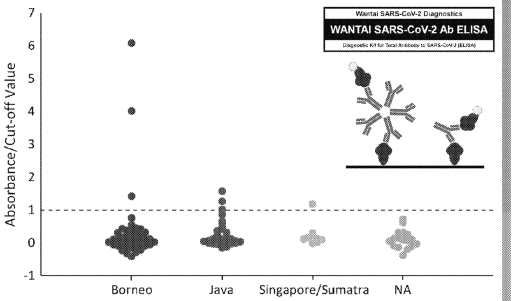


Fig. 5. Serology results for 168 samples. Dotted line represents seropositivity threshold and yellow bar indicates borderline seropositivity. All samples tested in duplicate with mean values represented. Double-antigen bridging assay concept for total antibody detection at upper right.

Table 1. Summary of individuals from each location with respective number of seropositive individuals and samples. Borderline omitted.

Origin	# individuals (seropositive)	# samples (seropositive)
Borneo	59 (2)	101 (3)
Java	15 (3)	39 (3)
Singapore/Sumatra	8 (1)	9 (1)
NA	7 (0)	19 (0)
Total	**89 (6)**	**168 (7)**

Discussion

- Seropositive individuals (all from the 2018 HK seizure) appear to originate from multiple wild populations, indicating that natural exposure to SARSr-CoVs may be common due to the shared ecology of pangolins, bats, and potentially other host species, or this may indicate infection acquired during the illegal trafficking of these animals.
- Our continued work aims to characterize the virome of these animals using metagenomic/transcriptomic approaches.
- Further investigation of wildlife trade networks is needed to identify potential poaching hotspots & trafficking hubs for intervention.
- Targeted surveillance efforts are needed to detect emerging pathogens which may be spread geographically through the illegal trade in wildlife products and live animals, risking exposure of humans and other animal species to novel pathogens.

Affiliations: [1]State Key Laboratory of Emerging Infectious Diseases, School of Public Health, The University of Hong Kong, Hong Kong SAR, P. R. China; [2]Guangdong-Hongkong Joint Laboratory of Emerging Infectious Diseases, Joint Institute of Virology (Shantou University/The University of Hong Kong), Shantou, Guangdong, 515063, P. R. China; [3]EKIH (Gewuzhikang) Pathogen Research Institute, Futian District, Shenzhen City, Guangdong, 518045, P. R. China; [4]Division of Ecology & Biodiversity, School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, P. R. China; [5]Ocean Park Corporation, Hong Kong SAR, P. R. China; [6]Centre for Immunology & Infection Limited, Hong Kong SAR, P. R. China; [7]HKU-Pasteur Research Pole, The University of Hong Kong, Hong Kong SAR, P. R. China; [8]Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore; [9]Research Center for Biology, Indonesian Institute of Sciences (LIPI), Jakarta, Indonesia; [10]Department of Zoology, University of Oxford, Oxford, United Kingdom; [11]Department of Pathobiology and Population Sciences, The Royal Veterinary College, London, United Kingdom; [12]Marie Bashir Institute for Infectious Diseases and Biosecurity, Charles Perkins Centre, School of Biological Sciences and Sydney Medical School, University of Sydney, Sydney, New South Wales 2006, Australia; [13]Laboratory of Data Discovery for Health Limited, Hong Kong SAR, P. R. China; *correspondence (tiylam@hku.hk)

References: Lam et al. (2020). Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature*.; Nash et al. (2018). Conservation genomics reveals possible illegal trade routes and admixture across pangolin lineages in Southeast Asia. *Conservation Genetics*.

Acknowledgements: Seized pangolin carcasses were donated for use in this study by the HK Gov Agriculture, Fisheries, and Conservation Department (AFCD) and by Kadoorie Farm and Botanic Garden. This work was supported by Research Impact Fund (R7021-20) University Grants Committee; the Health and Medical Research Fund (COVID190223), Food and Health Bureau, The Government of the Hong Kong Special Administrative Region; Guangdong-Hong Kong-Macau Joint Laboratory Program (2019B121205009); and the National Key R&D Program (2017YFE0190800).

From: Peter Daszak [b6]
Sent: 8/17/2021 12:35:49 PM
To: Morens, David (NIH/NIAID) [E] [b6 b6]; Jason Gale [j.gale@bloomberg.net]
CC: b6; Garry, Robert F [b6 b6]; b6
Subject: Nicholas Wade's piece today in Bull Atom. Sci.

Nicholas Wade's at it again – another miserable innuendo-filled attack on science and the process by which scientists email, talk and come to decisions. Nothing new of course, just trash journalism published in a pseudo-scientific journal. Pretty shameless smear on some of us – no facts of course, no sue-able accusations, just snipey comments and excerpts from Social Media. It's as if we can't now be scientists and actually colleagues who break bread and share a glass any more.

https://thebulletin.org/2021/08/how-covid-19s-origins-were-obscured-by-the-east-and-the-west/

The irony is in the ending – he claims the lab leak is an albatross hanging around China's neck that they won't be able to shake off until they open up the lab videos, books, freezers etc. The truth is his and others continued attacks are what put the albatross there in the first place. Does anyone really believe that if China gave access to all the above and no further evidence was found, that these folks would simply say "OK, it wasn't a lab leak after all"!!!

Cheers,

Peter

How COVID-19's origins were obscured, by the East and the West

By Nicholas Wade | August 17, 2021

A bus carrying a team of experts from the World Health Organization departs an airport in Wuhan on Jan. 14, 2021, after arriving in the Chinese city to investigate the origins of the coronavirus pandemic. That investigation is widely seen as having been obstructed by Chinese authorities. (Photo by Kyodo News via Getty Images)

Some 20 months after the Covid-19 pandemic first broke out, its origins remain obscure. A vigorous campaign of concealment by the Chinese authorities is the principal reason. But China received considerable help, strange to say, from senior medical research officials in the United Kingdom and United States who mishandled and effectively derailed the initial inquiry into the virus's origins.

The mishandling began at a pivotal teleconference held on February 1, 2020. The organizer was Jeremy Farrar, director of the Wellcome Trust, a large medical research charity in London. News of the conference emerged with the release this June of emails from the office of Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID). Farrar supplied further information in his book *Spike*, published on July 22.

The conference was held to discuss the unanimous view of a group of virologists that the SARS2 virus had been manipulated in a lab. Yet within a few days of the meeting, the virologists abruptly reversed their conclusion. The meeting's participants were later involved in two letters to scientific journals that stated the virus must have emerged naturally and that condemned any suggestion of manipulation as a conspiracy theory. These two letters, to *The Lancet* and *Nature Medicine*, shaped the views of the mainstream media for more than a year.


THE LANCET

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CORRESPONDENCE | VOLUME 395, ISSUE 10225, P397-398, FEBRUARY 01, 2020

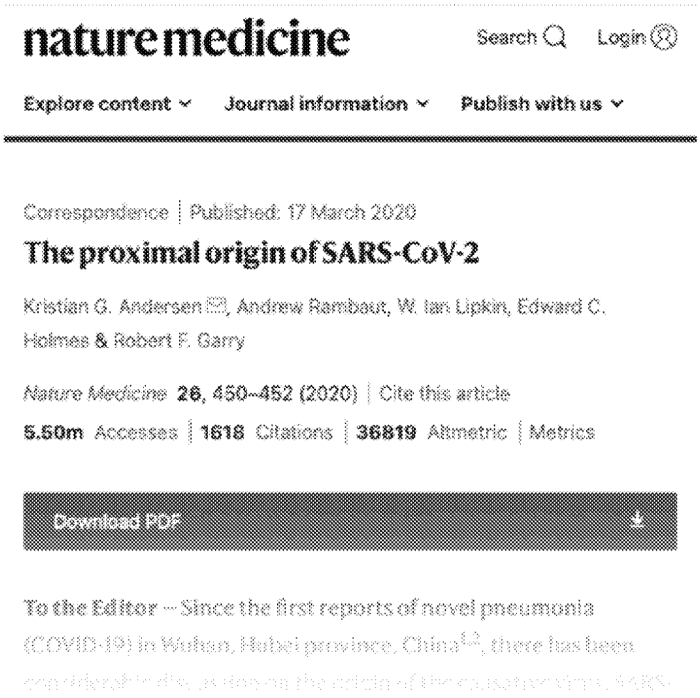
Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19

Charles Calisher ✉ • Dennis Carroll • Rita Colwell • Ronald B Corley • Peter Daszak • Christian Drosten • Luis Enjuanes • Jeremy Farrar • Hume Field • Joan Gibbons • Alexander Gorbalenya • Bart Haagmans • James M Hughes • William B Karesh • Gerald T Keusch • Sai Kit Lam • Juan Lubroth • John S Mackenzie • Larry Madoff • Jonna Mazet • Peter Palese • Stanley Perlman • Leo Poon • Bernard Roizman • Linda Saif • Kanta Subbarao • Mike Turner • Show less

Published: February 15, 2020 • DOI: https://doi.org/10.1016/S0140-6736(20)30418-9

PlumX Metrics

We are public health scientists who have closely followed the emergence of 2019 novel coronavirus disease (COVID-19) and are deeply concerned about its impact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Correspondence | Published: 17 March 2020

The proximal origin of SARS-CoV-2

Kristian G. Andersen ✉, Andrew Rambaut, W. Ian Lipkin, Edward C. Holmes & Robert F. Garry

Nature Medicine **26**, 450–452 (2020) | Cite this article

5.50m Accesses | **1618** Citations | **36819** Altmetric | Metrics

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To the Editor – Since the first reports of novel pneumonia (COVID-19) in Wuhan, Hubei province, China[1,2], there has been

Letters published by *The Lancet* and *Nature Medicine* in early 2020.

Even today, no one can say for sure whether the SARS2 virus emerged naturally or escaped from a lab. Much less could anyone have been sure back then. If the conferees had stuck to known facts, they would have left the question open to the two hypotheses, and the full exploration of the virus's origins might not have been sidetracked for over a year.

More significant, the Chinese government would have found it much harder, if not impossible, to manipulate the World Health Organization (WHO) into setting terms of reference that favored China's obstructive goals and kept WHO inspectors who visited China this February from accessing records vital to understanding the origin of the pandemic. China now insists those terms of reference cannot be changed, blocking further investigation into the origin of the SARS2 virus. "The two groups that produced the infamous letters in *The Lancet* and *Nature Medicine* paved the way for the Chinese government and helped enormously to facilitate all of that," says Milton Leitenberg, an arms control expert at the University of Maryland. The reversal of the virologists' conclusion about SARS2's artificial origin is thus a matter of some significance.

At 10:32 p.m. on the evening before the February 1 conference, Fauci had received an electrifying memo from Kristian G. Andersen, a virologist at the Scripps Research institute in California. Andersen reported that the virus seemed to be man-made. "The unusual features of the virus make up a really small part of the genome," he wrote, referring presumably to a genetic component known as a furin cleavage site, which greatly enhances the virus's infectivity, "so one has to look really closely at all the sequences to see that some of the features (potentially) look engineered."

From: Kristian G. Andersen (b) (6)>
Sent: Friday, January 31, 2020 10:32 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b) (6)
Cc: Jeremy Farrar (b) (6)>
Subject: Re: FW: Science: Mining coronavirus genomes for clues to the outbreak's origins

Hi Tony,

Thanks for sharing. Yes, I saw this earlier today and both Eddie and myself are actually quoted in it. It's a great article, but the problem is that our phylogenetic analyses aren't able to answer whether the sequences are unusual at individual residues, except if they are completely off. On a phylogenetic tree the virus looks totally normal and the close clustering with bats suggest that bats serve as the reservoir. The unusual features of the virus make up a really small part of the genome (<0.1%) so one has to look really closely at all the sequences to see that some of the features (potentially) look engineered.

We have a good team lined up to look very critically at this, so we should know much more at the end of the weekend. I should mention that after discussions earlier today, Eddie, Bob, Mike, and myself all find the genome inconsistent with expectations from evolutionary theory. But we have to look at this much more closely and there are still further analyses to be done, so those opinions could still change.

Best,
Kristian

A key email from Kristian Andersen to Anthony Fauci.
Andersen went on to note that "after discussions earlier today, Eddie, Bob, Mike and myself all find the genome inconsistent with expectations from evolutionary theory"—meaning that, in their unanimous view, the virus didn't come from nature. "Those opinions could still change," Andersen added. Eddie is Edward C. Holmes of the University of Sydney. Bob is Robert F. Garry of Tulane University. Mike is Michael Farzan at Scripps Research.
The message sent Fauci into a whirlwind of activity. "You will have tasks today that must be done," he emailed his deputy director, Hugh Auchincloss, two hours later at 12:29 am on February 1. One of these urgent tasks evidently concerned NIAID funds that had been passed, via the EcoHealth Alliance of New York, to Zhengli Shi, China's leading bat virus expert, at the Wuhan Institute of Virology. Fauci was doubtless anxious to check whether his agency's funding of Shi's work had complied with US law, which banned funding gain-of-function research from 2014 to 2017 and required it be reported to a government panel thereafter. "Gain of function" refers to research in which a pathogen's ability to cause disease is enhanced. Auchincloss replied a few hours later that efforts were underway to ascertain "if we have any distant ties to this work abroad."
Meanwhile Farrar, who had independently heard of the Andersen team's conclusion from Holmes, says in *Spike* that he arranged a teleconference set for 7 p.m. London time, convenient for both Washington and Australia, where Holmes was based.
The participants included Fauci and possibly his nominal boss, Francis Collins, the director of the National Institutes of Health. (Farrar says in *Spike* that Collins was present, but Collins's name is not on the list of invitees in the Fauci emails.) Officials on the UK side were Farrar and Patrick Vallance, the chief scientific adviser to the UK government.

The others were mostly virologists, including Andersen, Holmes, and Andrew Rambaut of the University of Edinburgh.

Far from being selected at random, the conferees were associated through a complicated web of relationships, a sort of virologists' old boy network that included senior medical officials in China. Farrar was well acquainted with George Fu Gao, the head of China's counterpart of the US Centers for Disease Control and Prevention. He has described Gao as an "old friend," who in fact had called a month earlier, on December 31, 2019, to tell Farrar about the initial cases in Wuhan of what turned out to be Covid-19. Farrar says in his book *Spike* that on the weekend of the teleconference, he called another highly placed Chinese official, Chen Zhu, China's minister of health from 2007 to 2013, to tell him of "rumours that the novel coronavirus could be the result of a lab accident." As for Holmes, he has published many papers both with Farrar and with Gao and has been a guest professor at Gao's CDC from 2014 until 2020.

Because of these varied connections, it seems likely that Chinese authorities knew about the conference almost from the moment it occurred and, if so, would have had the opportunity to influence deliberations that followed it.

At the conference there was a notable imbalance of power between the virologists and the officials. Fauci and Farrar together control a large portion of the funds available for virological research in the Western world. A virologist keen to continue his career would be very attentive to their wishes. Two of the conference participants had multimillion-dollar grant proposals under final review with NIAID at the time of the call.

Almost all references to what was discussed at the teleconference have been redacted from the Fauci emails under exceptions to the Freedom of Information Act. The conference would doubtless have included explanation from Andersen and Holmes as to why they had concluded the previous evening that the virus had been altered through laboratory manipulation. "Kristen and Eddie have shared this and will talk through it on the call," Farrar writes in one of the Fauci emails.

RELATED:
The known knowns, known unknowns, and unknown unknowns of COVID-19

From: Jeremy Farrar (b) (6)>
Sent: Saturday, February 1, 2020 1:13 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b) (6)>; Patrick Vallance (b) (6)
Cc: Drosten, Christian (b) (6); Marion Koopmans (b) (6)>; R.A.M. Fouchier (b) (6); Edward Holmes (b) (6) Andrew Rambaut (b) (6) Kristian G. Andersen (b) (6); Paul Schreier (b) (6); (b) (6); Ferguson, Mike (b) (6); Collins, Francis (NIH/OD) [E] (b) (6)
Subject: Re: Teleconference

Kristen and Eddie have shared this and will talk through it on the call. Thank you.

Hope it will help frame the discussions.

A Jeremy Farrar email about the teleconference.
Whatever was said at the meeting, it was followed by a remarkable and almost immediate about-face. By at most three days later, Andersen had executed a 180 degree turn in his views about the virus. In an email of February 4, 2020 to Peter Daszak, president of the EcoHealth Alliance, which had directed NIAID funds to Shi, Andersen wrote, “The main crackpot theories going around at the moment relate to this virus being somehow engineered with intent and that is demonstrably not the case.” The email was obtained by U.S. Right to Know, an investigatory group.
Participants have not explained what was said at the meeting or found in the days immediately following it to induce the change of mind. Media offices at the Wellcome Trust and NIAID declined to comment on this article. Andersen, Holmes and Rambaut did not reply to emails seeking an account of the reversal.
Farrar, not Fauci, seems to have been the leader in the teleconference’s deliberations. “This is not my area of expertise so I have backed off and am leaving it all to Jeremy,” Fauci wrote on February 13 to a CDC official. US officials were not to take the lead in this pivotal inquiry.

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 13 Feb 2020 22:36:17 +0000
To: Messonnier, Nancy (CDC/DDID/NCIRD/OD)
Subject: RE: NAS

Nancy:

The official USG group will be convened by NAS. Bob Kadlec is the person with direct knowledge of that. In addition, there is an ad hoc group informally led by Jeremy Farrar of Wellcome Trust. This group has about 15 people, all of whom are highly respected scientists, mostly evolutionary biologists who are convening by e-mail and conference calls (I have been on 2 of these calls since Jeremy invited me) to look at all of the bat, pangolin and human coronavirus sequences to try and determine the evolutionary origin. This is not my area of expertise and so I have backed off and am leaving it all to Jeremy.

Best,

Tony

Fauci's "leaving it all to Jeremy" email.

Farrar had a direct hand in the two letters that went out to the *Lancet* and *Nature Medicine*. He was a signatory of the *Lancet* letter, a draft of which Daszak, the organizer of the letter, began circulating just five days after the conference. The letter sought to squelch all discussion of the possibility that the virus had escaped from a lab by deriding it as a conspiracy theory. When Daszak wrote an article in the *Guardian* elaborating on the same theme, Farrar promoted it with a tweet, saying "as always worth reading @PeterDaszak."

Farrar also recruited the five authors who drew up the *Nature Medicine* letter, his spokesman told the writer Ian Birrell. (The spokesman referred to the *Lancet* letter but evidently meant the *Nature Medicine* letter, which has five authors.) The *Nature Medicine* letter, accepted on March 6 and published on March 17, 2020, presented a detailed and influential case that the virus had emerged naturally from animals. Its authors were Andersen, Rambaut, W. Ian Lipkin, Holmes and Garry.

In his book *Spike*, Farrar portrays the events between the February 1 conference and publication of the two medical journal letters as a judicious process in which he held an agnostic view and played no role other than asking questions. "On a spectrum if 0 is nature and 100 is release—I am honestly at 50!" Farrar says he emailed to Fauci and Collins a day after the conference. But if that were honestly so, he fails to explain his switch from 50 to 0 when signing the *Lancet* letter a few days later.

According to *Spike*, it wasn't until March, "after the addition of important new information, endless analyses, intense discussions and many sleepless nights" that Andersen and his four fellow virologists "were ready to pronounce on the origins of the novel coronavirus." Why then was Andersen, the virologists' leader, ready to pronounce just 3 days after the

conference that lab release was a conspiracy theory? Farrar's account does not match with the available facts.

Nor does Andersen's. In a June interview with the *New York Times,* he painted a picture that includes a change of mind on the possible engineering of the virus "in a matter of days, while we worked around the clock," and then that quick reversal being buttressed by drawn-out research.

"This is a textbook example of the scientific method, where a preliminary hypothesis is rejected in favor of a competing hypothesis as more data become available and analyses are completed," he said in a statement released by Scripps Research after his Jan 31 email to Fauci had become public. "I cautioned in that same email that we would need to look at the question much more closely and that our opinions could change within a few days based on new data and analyses—which they did," Anderson said in the *New York Times* interview. In that same interview, he also said that "more extensive analyses, significant additional data and thorough investigations to compare genomic diversity more broadly across coronaviruses led to the peer-reviewed study published in *Nature Medicine*."

These later data and analyses would not have been available three days after the conference, the date of Andersen's volte-face email to Daszak. And they would not have been available to Farrar as he signed the *Lancet* letter, which was published just 17 days after the teleconference.

More puzzling is Andersen's statement that in his preliminary studies "the genome of RaTG13, a SARS-related coronavirus found in bats, wasn't yet available." In fact its full sequence had been deposited by Shi in a data bank on January 24, 2020, a week before Andersen's report to Fauci, and Farrar says in *Spike* that he had seen the sequence by the time of the conference. RaTG13 is the closest known relative of SARS2, and the fact that it lacks the furin cleavage site found in SARS2 would have been a major reason for the Andersen group to suppose that this genetic element had been inserted into SARS2 in a lab.

Given his role in leading the February 1 teleconference, in presiding over the virologists' 180 degree change of views, and in arranging or influencing the *Lancet* and *Nature Medicine* letters, Farrar was evidently the driving force of the campaign to persuade the public that the SARS2 virus could not possibly have leaked from a lab. This was an unfortunate position for any scientist to take, given that he was assuring the public of something he could not be sure was true.

Farrar now says in *Spike* that, although natural emergence is more likely, "nobody is yet in a position to rule out an alternative." But his campaign sought very vigorously to do exactly that. "We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin," Farrar and his cosignatories wrote in the *Lancet* on February 18, 2020. This lapse in scientific judgment reflects also on the other senior participants in the conference who saw what was happening but apparently took no steps to insist that scientific truth should take precedence over unjustifiable professions of certainty.

RELATED:
Caltech's David Baltimore discusses the debate over origins of SARS-CoV-2

The decision to quash any notion of lab escape seems to have brought relief all round. At least until the tide of opinion began to change a year later, Fauci and Collins didn't have to endure unpleasant questions about why they had been funding hazardous research in minimally safe conditions at the Wuhan Institute of Virology.

"I just wanted to say a personal thank you on behalf of our staff and collaborators, for publicly standing up and stating that the scientific evidence supports a natural origin for COVID-19 from a bat-to-human spillover, not a lab release from the Wuhan Institute of Virology," Daszak emailed Fauci after a White House press briefing on April 17.

In August 2020 the NIAID announced it would award $82 million over five years to 10 participants in its new network for detecting infectious diseases. Among the lucky winners: Daszak, Andersen, and his associate Robert Garry.

The perturbing lab leak theory raised by Andersen and his colleagues on January 31, 2020 had been safely laid to rest. Farrar and his colleagues had succeeded in the one goal also pursued by the autocrats in Beijing, that of suppressing discussion of whether the SARS2 virus might have escaped from the Wuhan lab.

Beijing's way of squelching inquiry about the virus was, unintentionally, somewhat more obvious than the methods used in London. Chinese authorities tried so hard to stamp out information about the virus's origin that they left a rather clumsy trail of footprints pointing to where they didn't want people to go.

One of the more informative suppressions of data was the closure of China's main database on bat and other viruses. Zhengli Shi, China's leading expert on bat coronaviruses, told the BBC that it was taken off line because of numerous hacking attempts. It's conceivable that in January 2020, after the pandemic broke out, people without authorized access might have tried to hack into the database. But in fact the database went off line on September 12, 2019. Who would have wanted to hack into a bat virus database back then? More likely, that is the date at which Chinese authorities realized they had a virus escape problem.

In February of last year, President Xi referred to the need to ensure biosafety and biosecurity, and the Chinese Communist Party followed by tightening up their biosafety rules in October 2020, accelerating a long planned revision. "To me, the fact that the CCP enacted a major series of laboratory biosafety regulations this past year is an indication that China's political leadership believes that a lab accident is likely to have caused the pandemic," says a biosurveillance expert who has monitored China's disease outbreak reporting for the past two decades.

Major clues as to what happened are evident in Shi's various pronouncements about the virus. The omissions and untruths in these statements are specific enough to outline the very body of facts the censors have sought to conceal.

Wuhan Institute of Virology researcher Shi Zhengli spoke about SARS during a Yixi event (comparable to a TED talk) on June 23, 2018. (Yixi video)

In looking at Shi's questionable statements, it's only fair to keep in mind that they may have been compelled. Her train of deceptions began immediately after the genetic sequence of the SARS2 virus was published on January 10, 2020, by Yong-zhen Zhang of Fudan University, against the wishes of Chinese authorities who subsequently closed his lab for a time. Where did this strange new virus come from? Shi wanted or was told to establish SARS2's pedigree as a bat virus, with an ancestry analogous to that of SARS1, the bat virus that caused an epidemic in 2002. In an important paper published on February 3, 2020, Shi reported that the genome of SARS2 is 96 percent similar to that of a bat virus, RaTG13, "which was previously detected in [the bat species] *Rhinolophus affinis* from Yunnan province."

Shi neglected to mention a salient difference between the two viruses, namely that SARS2 possessed a furin cleavage site and RaTG13 did not. Alina Chan, of the Broad Institute in Cambridge, has likened the omission to describing a unicorn by reporting all its horse-like features, but neglecting to mention the horn. Evidently the Chinese authorities were highly sensitive to the furin cleavage site's presence.

Shi also failed to say when and where she had discovered the RaTG13 virus, surely important data for the closest known relative of SARS2. In fact, she had found RaTG13 in 2013, in an abandoned mine in Tongguan—the same place where six miners had fallen sick the year before. At that time, she analyzed just one of its genes and reported the virus under another name, BtCoV/4991. Daszak, the holder of her NIAID grant, told the London Times, perhaps on misinformation from Shi, that the virus was then thrown in a freezer and forgotten about until 2020. This was untrue—the virus was of much greater interest. Shi had analyzed its full genome, probably by 2018, but did not publish it. It was only when she needed a bat virus pedigree for SARS2 that Shi released information about the

virus, which she now renamed RaTG13. The fact that BtCoV/4991 and RaTG13 were one and the same was discovered by Monali Rahalkar and Rahul Bahulikar, two internet-sleuthing researchers in Pune, India.

It's not acceptable practice among scientists to report something already published under a different name as if were new. Shi's February paper had the goal of portraying SARS2 as just another bat virus while concealing the true provenance of its closest known relative—a cave known to harbor lethal viruses.

When RaTG13's connection to the Tongguan mine was pointed out, Shi still tried to conceal the lethality of the mine's bat viruses, saying the miners had died of a fungus infection. This untruth was corrected when a master's thesis by a Chinese doctor, Li Xu, was unearthed by the internet sleuth who calls himself TheSeeker268. The thesis reported that the miners had died of a SARS-related virus with symptoms identical to those of Covid-19 and with CT scans similar to those of Covid patients. The only evident difference between that virus's effects and those of SARS2 is that the miners' virus was not readily transmissible from one person to another.

The specificity of what Shi's statements concealed—the furin cleavage site, RaTG13's identity with BatCoV/4991, the latter's origin in the mine where six miners were infected, the death of the three miners from infection by a virus with symptoms very similar to those of SARS2—all point to the involvement of these elements in a scenario the Chinese authorities are determined to cloak. That scenario may have been the storage or generation of the SARS2 virus in Shi's lab from one of the many viruses recovered from the Tongguan cave.

The Chinese government's persistent stonewalling and Shi's pattern of evasions do not amount to proof that the SARS2 virus escaped from her lab. But they seem less like the actions of innocent people and more like attempts to cover up a fatal accident, one that has caused the deaths of maybe 10 million people and counting.

Perhaps proof will one day emerge that the virus emerged naturally. If not, the likelihood that SARS2 escaped from a researcher's laboratory is an albatross that will hang round China's neck in perpetuity. China's only hope of release from this terrible encumbrance lies in opening its laboratory doors and either establishing its innocence or admitting its fateful error.

Cheers,

Peter

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From: Morens, David (NIH/NIAID) [E] b6
Sent: Sunday, August 15, 2021 6:26 PM
To: Jason Gale <j.gale@bloomberg.net>
Cc: b6; Garry, Robert F b6
Subject: RE: feedback on wild animals

Jason, not sure whose office politics is worse, but there is many a day I'd rather be in Australia, even if I had to drink that awful beer Fosters. (To quote Grouco Marx as he help up a glass of questionable beer: "last time I saw something like this, they had to shoot the horse")…. But I do have fond memories of Australia, and two of my closest freiends, both ex-students of mine, are out in Perth, the only large city there (if oy can call it that) I have never been, although it's high on my bucket list if this damn pandemic ever ends. He's an American public health physician and she's an Australian-Tongan PhD virologist, both wondeful smart people.

David

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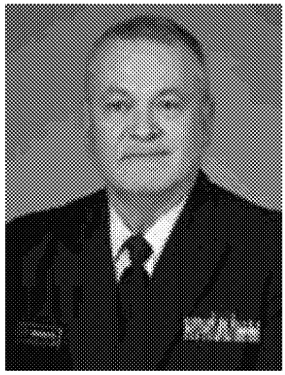

From: Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
Sent: Saturday, August 14, 2021 7:54 PM
To: Morens, David (NIH/NIAID) [E] b6
Cc: b6; Garry, Robert F
b6
Subject: Re: feedback on wild animals

Thanks, David.
I just got off a video conf call with the current editor in NY (the one asking all the latest questions). He's very nice and I feel like he gets its. He thinks it's a fascinating story.
Problem we have in journalism is that there are some people who aren't interested in actual journalism and telling stories; they want to climb to the top and manage people. I rejected the latter route 10 years ago, but am so grateful that I still get to meet so many remarkable people, visit cool places and see all aspects of humanity.
A difficulty working for a NY-based organization is that it's assumed that all the best people are in NYC, so being in Melbourne (where I need to be to be close to my kids in Adelaide), is that folks consider Australia a backwater and, by association, I mustn't be all that important/valuable.
But my new role as "global biosecurity czar" is helping somewhat.
Ahhhhh... Office politics!

From: b6 At: 08/15/21 09:03:03 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: b6;
b6
Subject: Re: feedback on wild animals

Jason, all I can say is that it must be harder to be a journalist than a scientist…. I had no idea someone at your level would get pushed back.

Usually i get from one to 4 reviewer responses to a ms. and three of those are out to lunch. Occasionally i get a reviewer who really understands the work: half of those are helpful, the other half are trashers.

Maybe it's like being in the government where i am: there is endless push back, but the push-backers are brainless idiots who don't know what they are talking about.

Do you ever get to a point where the editors leave you alone on the science? Or do they all think they are science geniuses? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

> On Aug 14, 2021, at 16:05, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:
>
> Thanks, Bob. In journalism, getting to the truth even when it's genuinely and actively pursued, can be a tortuous process!
>
> ----- Original Message -----
> From: Robert F Garry b6
> To: JASON GALE, b6,
> b6
> At: 08/15/21 01:45:11 UTC+10:00
>
> Looking forward to this important article. Truth is stronger than fiction.
>
> **From:** Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net>
> **Sent:** Saturday, August 14, 2021 6:41 AM
> **To:** b6
> b6
> Garry, Robert F b6
> b6
> **Subject:** Fwd:Re:feedback on wild animals
>
> External Sender. Be aware of links, attachments and requests.
>
> Hi guys, in case you have nothing better to read over the weekend, this is some of the dialog I am having with editors in the U.S. Essentially, my response to questions from editor #6.
> I thought I could claim victory when it looked like the story could be published this morning, but the editor of Businessweek objected and thinks he can make it better

with another revision and an ETA of Tuesday.
Sigh.
Jason

From: Jason Gale (BLOOMBERG/ NEWSROOM:) At: 08/14/21 16:36:58 UTC+10:00
To: Cristina Lindblad (BLOOMBERG/ NEWSROOM:) , Eric Gelman (BLOOMBERG/ NEWSROOM:)
Cc: Joel Weber (BLOOMBERG/ NEWSROOM:)
Subject: Re:feedback on wild animals

> Hi.
> Thanks for the feedback and the questions, which I have tried to answer in detail.
> It's often easier to have a conversation over Nexi or the phone to explain nuanced information, or details that are clear to me, but might not be to someone coming at this fresh, but that's tricky with the time difference.
> When you look back at what's happened here, it seems that in trying to deflect blame for the pandemic, which became increasingly vicious as the cataclysmic nature of the Covid-19 pandemic unfolded around March-April 2020, China tried to conceal a very obvious, very plausible source of the pandemic: it's flourishing wildlife trade (worth about $90 billion in 2016).
> It was an obvious cause of the pandemic because an almost identical scenario triggered an international outbreak caused by a very similar coronavirus (SARS) in 2003-04. But in attempting to cover up the wildlife trade, and making like there were never any wild animals being sold in Wuhan's wet markets, things began to backfire on China; questions were raised about the nearby lab studying these coronaviruses. The more geopolitical, heated and vicious the arguments and accusations became, the less cooperative China became. In

response, the more intent/adamant some groups have become in their belief that China is covering up a lab-leak. It's become a vicious circle. If China isn't coming clean on the wild animals, what else is it trying to hide?? China's defensiveness means we may never get the cooperation needed to find the answers. If China had been honest and transparent about the wild animals in the wet markets, it might not be in this mess now.

Anyway, here are my response to your queries in green.

By the way, I wonder if the current headline:
Delayed Paper Gives Credence to Wuhan Market Covid Origin Story
doesn't convey much more than what we had 2 months earlier when we reported Xiao's findings: China Markets Sold Mink, Civets, Stoking Natural Origins Theory
Perhaps Obscured China Paper Scuppered Chance to Trace Covid Origins
would hits "China", "Covid" and "Origins" in a way that won't alienate people who already believe it was a lab-leak

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According to the report, minks, civets, raccoon dogs and other mammals known to harbor coronaviruses were sold in plain sight for years in shops across the city, including the now infamous Huanan wet market, to which many of the earliest Covid cases were traced. The evidence collected over 30 months by **Xiao Xiao** a I'D NAME HIM HERE [[i don't know that naming a researcher no one has ever heard of adds much]] researcher working at a lab affiliated with China's Ministry

of Education was hastily drafted into a manuscript and submitted to a scientific journal [Joel, we cannot name the first journal they delivered it to because the authors decline to give us the name, saying it may affect their future chances of being published] in February 2020, just weeks before the outbreak was declared a pandemic.

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While the study received wide attention when it was eventually released by a different publisher [Publisher (Springer Nature) and publication (Scientific Reports) are different. Might add confusion DO YOU WANT TO ADD NAME HERE?], its long and torturous journey to publication gave **Chinese officials an opportunity to weave alternative narratives in which the virus may have come from abroad, even from a U.S. Army biological research facility.** [the stuff in bold is not contested and we go on to show how that happened, documenting with links when available]I TWEAKED WORDING HERE BUT THIS STILL ISN'T QUITE RIGHT. IF THE REPORT HAD BEEN PUBLISHED EARLIER CHINESE OFFICIALS COULD STILL HAVE DONE WHAT THEY DID—THEY COULD HAVE JUST SAID THE REPORT WAS SHODDY, OR WHATEVER, AND THEY ALSO COULD HAVE JUST IGNORED IT. I disagree. The evidence that Xiao et al provide was meticulously documented and supported by photographs that would have been difficult/impossible for China to dismiss (as older published photos and media reports had been). What's more, Newman understands that Xiao collected blood-sucking ticks from the wild animals he studiously cataloged. His frozen tick samples could be tested for

blood/antibodies/virus, which could be extremely helpful in identifying infected species PRIOR to December 2019. The WHO team knew nothing of this, so couldn't have asked China for this research or any results, had they actually done the research. The delay in the publication of Xiao's paper delayed the evidence that there were live animals sold in the Huanan market. The WHO researchers couldn't have asked about tests on wildlife that ostensibly were never there. Likewise, Chinese authorities couldn't have done the tests on animals that didn't exist. The problem is that in January and February 2020, it was widely assumed the animals HAD been there and that the necessary tests and tracing of animals (the sampling of animals on farms they were raised on, testing of farm workers, animal hunters, transporters and traders **had all been done** by researchers in China -- the very things that ultimately led to the discovery of the origins of SARS and of MERS viruses. **None of these things were done** (or at least, there is nothing publicly available to show that they were done) **because the animals "weren't there"**. Now that we all know they were there, China as lost considerable face. The issue has become so political than there is much less/?no willingness to cooperate and conduct the additional research that the WHO-led team recommended. Ideally, Xiao's ticks should be studied, but it's doubtful that will now happen (I wouldn't be surprised if he's been ordered to incinerate them!) Hope this is clear.

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An international team of experts convened by the WHO traveled to

Wuhan earlier this year to seek answers—a trip that might have yielded different results if the scientists had known about the work of Xiao Xiao, a virologist whose roles straddled epidemiology and animal research at the government-funded Key Laboratory of Southwest China Wildlife Resources Conservation and at Hubei University of Traditional Chinese Medicine.'MIGHT HAVE YIELDED DIFFERENT RESULTS' SEEMS HIGHLY SPECULATIVE. WHAT RESULTS DID IT YIELD? I'D CUT THIS GRAF

As mentioned above, the WHO-convened team of researchers was told by market authorities, vendors and regular market visitors that there were no live animals sold in the Huanan market. That undermined completely the premise that the Huanan market was the kind of place where live animals from different species are stacked in cages, with urine and fecal material drips from one cage to the one below it, where there are splatters of blood and guts from animals, and lots of potential for the spread of diseases from one species to another (including Homo sapiens). Instead, the market (and one other one known to have been selling live wild animals in 2019) was presented as selling only frozen wild animals and aquatic species and things unlikely to be the source of SARS-CoV-2. The WHO researchers were told there were frozen ferret badgers and other wildlife found in freezers. Some of the carcasses actually came from Yunnan, the province where the closest coronavirus related to SARS-CoV-2 was found in bats -- thus establishing a potential route from Yunnan to Wuhan in wildlife. That was actually important. When the researchers showed their Chinese counterparts

photos of caged raccoon dogs taken in the Huanan market by Prof. Edward Holmes five or six years earlier, they were told by Chinese scientists that the photos may have been faked, and that the market had ceased selling such live animals anyway. The WHO researchers saw no evidence (empty cages, animal pelts, etc) to dispute what the Chinese scientists told them, although they did smell "animals" -- but were told they were smelling rotten meat, sewage etc. Three people associated with the WHO-led mission told me that they didn't believe the information they were given by anyone associated with the market. But since there was no evidence to the contrary, they were unable to push the Chinese scientists further on this. **The fact that the mission concluded that frozen, not live wild animals were sold in the market undermined the thesis that Covid resulted from an animal spillover.** And the absence of strong evidence pointing to a spill over from wild animals to humans made the lab-leak theory look, in relative terms, more plausible.

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Six months and two revisions later, the journal's publishers rejected the paper. "They did not think it would have widespread appeal," says Newman, who declined to name the publication [do you want us to say why he won't name it? I don't think it's necessary. There's no upside for scientists to make a publisher look bad (most journals are published by a handful of publishers)] "It caused us, especially our Chinese co-authors, concern that these data would not be taken seriously."

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The manuscript underwent a third revision to include data on China's pangolin trade networks (an earlier study, later contested, had implicated pangolins in the virus's spread to humans)WHO ASKED THAT THIS INFO BE INCLUDED? (I did, because we're trying to ezplain here why WHO missed the significance of Xiao's evidence. The WHO was slammed by other papers being submitted PLUS Xiao's paper had a weird title. That title seemed relevant to the authors back in January-February 2020, when pangolins were considered a possible SARS-CoV-2-spreading culprit. But in October 2020, pangolins were off the hook, so the title would have seem irrelevant/unimportant at first glance at WHO). It was then sent to the online journal *Scientific Reports*.

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The China-based researchers had reason to be cautious. In February 2020, the China Center for Disease Control (CCDC lets not use "CCDC". It's not commonly used like the U.S. "CDC" is, and it's going to force readers to go back and figure out what the "CCDC" is) prohibited scientists working on Covid-relate

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~~CCDC~~ Disease detectives arriving from Beijing (think this makes it clear that these are China's disease "feds" arriving) on the first day of 2020 ordered environmental samples to be collected from drains and other surfaces at the market. Some 585 specimens were tested, of which 33 turned out to be positive for

SARS-CoV-2. "All current evidence points to wild animals sold illegally," China CDC Director George Gao and colleagues wrote in the agency's weekly bulletin in late January. All but two of the positive specimens came from a cavernous and poorly-ventilated section of the market's western wing, where many shops sold animals.

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~~The information void kindled a raging political debate that's already caused a trade war between China and Australia, as nations demand to know how Covid emerged.~~ Australia in April 2020 called for a global inquiry into the origins of the pandemic, including China's handling of the initial outbreak. Days later, then-U.S. Secretary of State Mike Pompeo used part of his Earth Day message to call on China to close its wet markets to "reduce risks to human health inside and outside of China."

Based on a discussion today with someone with knowledge of the WHO-led mission to Wuhan in Jan-Feb 2021, a significant catalyst for China's defensiveness is the emergence of **claims for reparations** that extended from the finger-pointing at China's wet markets. These surfaced in April 2020, and have continued as recently as June 2021 (when Trump pushed for it again at a rally in the Midwest)

SO IS THIS THE POINT AT WHICH CHINA, WHICH SEEMINGLY ACCEPTED THE WET MARKET HYPOTHESIS, BEGAN TRYING TO CREATE A DIFFERENT NARRATIVE? (I think it was incremental. I believe China was embarrassed that its citizens were still buying wild animals in wet

markets to eat -- a well-known hazard for zoonotic disease transmission that China tried unsuccessfully to outlaw almost 20 years ago. But that embarrassment/humiliation morphed into rigid denial and obfuscation when governments began openly blaming the Chinese Communist Party and agitating for China to pay reparations for the pandemic. See these clips:

- USA Today: Blame the Chinese Communist Party for the coronavirus crisis: Coronavirus crisis proves communism is still a grave threat to the entire world. If Beijing had just been honest, the pandemic could be preventable. April 5, 2020
- Yahoo News: More than half of Americans think China should pay coronavirus reparations, poll shows April 9, 2020
- Voice of America: Americans Join Coronavirus Lawsuit to Make China Pay April 10, 2020
- Washington Post Opinion: China must pay reparations to Africa for its coronavirus failures April 16, 2020
- Reuters: In a first, Missouri sues China over coronavirus economic losses April 22, 2020
- Washington Post: Missouri is suing China over the coronavirus pandemic. It's the latest conservative gambit, April 22, 2020
- New York Post: Top German paper demands $165 billion coronavirus reparations from China April 22, 2020
- Attorney General Fitch Prepares to Sue China on Behalf of Mississippians April 22, 2020
- Newsweek: Trump on U.S. Seeking Compensation From

China Over COVID-19: 'We Have Not Determined the Final Amount' April 28, 2020

- The Guardian: Trump says China could have stopped Covid-19 and suggests US will seek damages April 28, 2020
- Intelligencer: Trump Thinks He Can Make China Pay for the Virus Like Mexico Paid for the Wall, April 30, 2020
- Washington Post: U.S. officials crafting retaliatory actions against China over coronavirus as President Trump fumes April 30, 2020
- Lawfare: Does China Really Owe the World Trillions of Dollars? May 7, 2020
- Fortune: Trump's demand that China pay coronavirus reparations evokes an ugly history May 8, 2020
- South China Morning Post: Why China won't be paying the West coronavirus reparations any time soon May 15, 2020
- Deccan Herald, India: Abhijit Bhattacharyya | Why China needs to pay reparations to the world June 4, 2020
- Why calls for reparations from China for coronavirus are an unfeasible distraction June 9, 2020
- Newsweek: Trump Demands China 'Pay Reparations' for COVID, Says $10 Trillion Not Enough June 12, 2021

In response, Geng Shuang, a spokesman for China's Foreign Ministry, denied "wildlife wet markets" existed in the country. Government researchersCAN WE BE MORE SPECIFIC? Twenty-two researchers from mostly nationally-funded laboratories (I think it's the Chinese equivalent of the NIH in the U.S.) and institutes attached to the Chinese Academy of Sciences) now dismiss

the market hypothesis completely. "SARS-CoV-2 could not have possibly evolved in an animal market in a big city and even less likely in a laboratory," they wrote in a paper released last month ahead of publicationWHERE/WHEN WILL IT BE PUBLISHED? It was released as a "pre-print" on a Chinese academic repository ahead of publication that appears to be managed/owned by the Chinese Academy of Sciences. Papers are usually released in pre-print form before they have been accepted for publication or peer-reviewed as a way of expediting public access to the information. In this case, there is no information to suggest if, where or when the paper will be published.

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A more recent paperBY WHOM? (China CDC's Gao and eight other scientists mostly from the Chinese of Academy of Sciences' institutes) contends that the virus may have been imported from multiple locations worldwide, including parts of Europe where mink are raised in areas inhabited also by horseshoe bats known to harbor coronaviruses. "The official narrative changed not because the evidence changed," says Robert Garry, a professor of microbiology and immunology at Tulane University's School of Medicine in New Orleans "A spillover from a wet market was what caused SARS, and, embarrassingly for China, those wet markets were never shut down." Garry is the co-author of one of the earliest papers on the origins of Covid but wasn't involved in the research on Wuhan's markets.

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I DON'T REALLY UNDERSTAND WHAT POINT THIS GRAF IS TRYING TO MAKE. This is intended to demonstrate the kind of gaslighting that has occurred. Some of the WHO-led researchers are veterinarians and zoologists -- they know what animals smell like, and could smell their lingering presence a year later, but were told essentially that it was impossible that they were smelling animals because **there "were no live animals there**".) The researchers noted a mixed smell of animals and disinfectant in some areas of the market, but they were told by the market's manager that they were probably smelling the lingering stench of rotten meat and sewage, according to a joint WHO-China report. [should we add "according to Laing Wannian etc here? Liang Wannian was the leader of the Chinese research team collaborating jointly with the WHO-led research team. The source for the above description are in the annexes to the official joint WHO-China report released at the end of March 2021.]

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Earlier the same day, the international research team visited Wuhan's larger Baishazhou market, where Xiao had regularly surveyed two sellers of live wild animals. Yet the group was told only frozen food, ingredients, and kitchenware were on offer there. Liang Wannian, an epidemiologist who led the Chinese experts collaborating with the WHO-convened team, says his group had no knowledge of Xiao's data either. [if the above information on what the delegation saw all comes from this same source, maybe we should include his name higher

up (The source for the description of what the origins researchers saw on Jan. 31, 2021, comes from the official 120-page joint WHO-China report and its 193-page annexes, not from Liang. Since we are essentially accusing China of concealing the information that Xiao documented, we asked Liang at a press conference in late July 2021 when the Chinese team first knew about Xiao's findings -- that the Huanan market and three others in Wuhan were selling live animals permissive to SARS-CoV-2 infection -- and what research China has done subsequently as a follow-up on this information? Liang gave a very long-winded response in Chinese in which he said essentially "we didn't have that information in January-February 2021 when the research team was in Wuhan". I think it's important we keep this to demonstrate that we have tried to ascertain what China knew/has done and have given the Chinese researchers the opportunity to respond. In addition, I have emailed China CDC Director George Gao at least twice and not received any response.]

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Among the earliest clusters of infections recorded in Wuhan, one involved three Covid cases among staff working at a stall in Huanan. One of the employees, a 32-year-old who fell ill on Dec. 19, traded goods back and forth between the Huanan and Baishazhou markets.
WHERE DOES THIS FACT COME FROM? NOT THE REPORT, RIGHT? WHEN DID IT FIRST BECOME KNOWN? This information was from the joint WHO-China report released in March 2021, however, the WHO-led researchers went to the Baishazhou market more as a demonstration by

the China team of what a perfectly functioning large food market looks like. The WHO-led team was oblivious to Xiao's research that showed there were at least two stalls in Baishazhou that had been selling live wild animals for human consuption. So this detail and its significance was lost on the WHO researchers at the time.)

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A confirmed case linking two markets that sold wild animals is "very intriguing,"says Stephen Goldstein, a research associate in evolutionary virology at the University of Utah in Salt Lake City. But tracing any contact the employee might have had with infected wildlife is impossible now that the animals are long gone.WOULD TIMELY PUBLICATION OF THE REPORT HAVE CHANGED THIS? It's unlikely that Xiao et al's paper would have been *published* soon after It was drafted in February 2020, but it could have been *released* as a pre-print ahead of publication and peer-review that same month. That would have **confirmed** what almost everyone had suspected: that there **was** a flourishing wildlife trade in Wuhan that provides a plausible pathway by which coronavirus-infected wildlife from Yunnan and beyond could have introduced the virus to the city, sparking the Covid outbreak. It's also possible that swift recognition of these potential wild-animal vectors could have allowed scientists to test them for the virus and for antibodies against the virus while they were still alive (perhaps not the ones from Huanan, but wildlife in the three other Wuhan markets). Because of the statements the Chinese authorities had been making in January and February

that strongly supported the animal spill over theory, it was assumed that this research was being done. Of course, the pandemic was raging then, so it would have been challenging. But, because of the denial by Chinese researchers that wild animals were being sold in Wuhan wet markets before the pandemic, there was never that level of follow up. At least, if there was, it was never made public.)

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"It seems to me, at a minimum, that local or regional authorities kept that informationWHAT INFORMATION? (that Wuhan had a flourishing live wild animal business going on in its wet markets before the pandemic) quiet deliberately," Goldstein says. "It's incredible to me that people theorize about one type of cover-up, but an obvious cover-up is staring them right in the face."

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From: Edward Holmes [b6]
Sent: 9/20/2021 6:16:06 AM
To: Jason Gale [j.gale@bloomberg.net]
CC: Peter Daszak [b6]; David Morens [b6]; Morens, David (NIH/NIAID) [b6]; Kristian G. Andersen [b6]; Wang Linfa [b6]; Garry, Robert F [b6]; Taubenberger, Jeffery (NIH/NIAID) [E] [b6]; b6
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Yes, it's very odd. I just can't follow it.

What I did think was interesting - as you note - is that they talk a lot of the market which I thought was totally off the table in China. Also, it's from Nanshan Zhong and I last time I heard him speak he was strongly pushing the frozen food idea.

Perhaps a shift?

Or could just be a wet Wednesday afternoon's ramblings.

PROFESSOR EDWARD C. HOLMES FAA FRS
ARC Australian Laureate Fellow

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T
E b6

On 20 Sep 2021, at 4:06 pm, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I thought the SARS-CoV-2 virus that emerged in Wuhan wasn't capable of infecting mice that weren't genetically engineered to express human ACE2? This paper, with its emphasis of meteorological factors, seems dodgy to me. But it's good to see research on the origins from researchers in China getting out, albeit it in an obscure journal.

From: b6 At: 09/20/21 15:55:39 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: b6,
b6

b6

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Although I can't quite tell if it is sane.

PROFESSOR EDWARD C. HOLMES FAA FRS
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T b6
E

On 20 Sep 2021, at 2:37 pm, Edward Holmes b6 wrote:

Just found this in an obscure journal.

Interesting it is Nanshan Zhong and interesting that there's a lot about the market….

PROFESSOR EDWARD C. HOLMES FAA FRS
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On 20 Sep 2021, at 10:52 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I did this podcast episode on bats and zoonoses at the start of 2020 with the help of Hume Field, Trevor Drew, Mark Schipp and Linfa. Still seems relevant today. https://podcasts.apple.com/nz/podcast/how-it-all-started-rebroadcast/id1440051086?i=1000504072911

From: b6 At: 09/20/21 10:17:32 UTC+10:00
To: Jason Gale (BLOOMBERG/ NEWSROOM:)
Cc: b6

b6

b6

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

100% agree.

PROFESSOR EDWARD C. HOLMES FAA FRS
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On 20 Sep 2021, at 10:16 am, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

Suspect geopolitics is the biggest impediment to finding an animal source in China, and the best remedy for this is to rebuild/strengthen r'ships with scientists in China.

From: b6 At: 09/20/21 10:12:48 UTC+10:00
To: b6
Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) , b6 ,
b6
Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

Just need to keep sampling, but that sampling ought to be broader.

We need something >99% similar across the whole genome.

PROFESSOR EDWARD C. HOLMES FAA FRS
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T
E b6

On 20 Sep 2021, at 9:59 am, Morens, David (NIH/NIAID) [E] b6 wrote:

Agree totally except your certainty that China is the ultimate source. Admittedly much data point in that direction but how can you be sure? d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 19:28, Edward Holmes b6 wrote:

It's not phylogenetics.

One thing is ascertainment bias which could be huge.

Second thing is to distinguish the long-term ecology of these viruses from the short-term emergence of the virus. These Laos viruses are the former. Clearly these viruses are commonplace in SE Asia. And I don't just think that bats and pangolins will be the only animals with SC2-like viruses. Virus ecology does not work like that. But this is not the same as determining the events that happened in Wuhan. To me, China still looks like the most likely source.

Third, I'm pretty certain that groups in China are sitting on more SC2-like viruses. If you sample bats you find them. It is striking to me that CCDC have published so little on this yet have supposedly sampled so many animals. That doesn't add up. Never discount the politics.

Professor Edward C. Holmes FAA FRS
The University of Sydney

On 20 Sep 2021, at 9:00 am, Morens, David (NIH/NIAID) [E] b6 wrote:

Eddie, please clarify, i don't « get » all the phylogenetic asumptions you guys understand, but can you put it in Isyman's terms? As you know, i have said repeatedly to look past Yunnan to all of SE Asia, as i have bennunconconvinced of the Yunnan centrality of all this, suspecting thAt the universe of these viruses crosses borders to include not only SW and S China but all of SEA.

If that is so, the implications ate huge: this is annintetnational problem demanding international cooperation. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 18:33, Edward Holmes b6 wrote:

Yes, good idea.

The receptor binding domain of some of these Laotian bats is so close to that of SARS-CoV-2 even some of the die-hard leakers are beginning to see the light…

This also effectively excludes that virus-receptor relationship was generated through lab passage, that the pangolin sequences were faked, and that this outbreak had anything to do with the Mojiang mine as a virus from a different country is now closer. That mine will go down in history as the reddest of herrings.

That said, I am a little worried about confirmation bias for the origin being bats from Yunnan/Laos/Cambodia. The more they find there, the more they sequence. But no doubt these Laotian samples are of huge significance. As are the Hubei civets.

<Screenshot from 2021-09-19 17-04-25.png>

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T
E **b6**

On 20 Sep 2021, at 7:52 am, Morens, David (NIH/NIAID) [E] **b6** wrote:

Yes, do it! This is important and i say modestly, game changing. The whole « origin » controversy needs to be rethought from the ground up

We have been too micro-focusing (as i have long said to hard push back) but the sarobecovirus and merbecovirus problems are geographically and virologically complex and require us to drop back and study the viral-host universe. Thst universe is huge, complicated , and holds surprises, in my view. d

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 19, 2021, at 17:36, Jason Gale (BLOOMBERG/ NEWSROOM:) <j.gale@bloomberg.net> wrote:

I'm planning to pull the threads Peter has so eloquently laid out into a story. Bob, Stephen, Joel (and Kristian), if you have time/interest to get on Zoom today, let me know. Thanks a lot. Jason

From: **b6** At: 09/20/21 07:31:51 UTC+10:00
To: **b6**
Cc: Jason Gale (BLOOMBERG/ NEWSROOM:) , **b6**,
b6,

b6

Subject: Re: Study from 2007 shows SARS-infected civets on farms in Hubei

nPeter, as i am perennially swamped with work that has nothing to do with COVID issues of importance, i am always catching up on reading the important stuff

Just now i poured a martini and-read word for word your "A strategy…" paper with first author Sánchez. Also Kevin and Lin-fa were coauthors. Wow!!!

This is dynamite and also beautifully written. I mean, Hemingway, Conrad, Nin, couldn't have written it better. Beautiful job and so important.

I think you need to promote this work, and emphasize that the conclusions are far reaching and a sort of call to arms.

Let us all keep pushing,
and keep our eyes on the
prize of getting to the bottom of it all
david

Sent from my iPhone
David M Morens
OD, NIAID, NIH

On Sep 18, 2021, at 12:05, Peter Daszak b6 wrote:

I put it all in a twitter thread while drinking coffee in my local diner (Saturday is "full English breakfast" day for me).

https://twitter.com/peterdaszak/status/1439236376776658945?s=21

No doubt ill be attacked by multiple lab leak aficionados but so be it - at least eddie,
Garry and Kristian won't see. The horrors of that…

Cheers,

Peter

Peter Daszak
(Sent from my iPhone)

President
EcoHealth Alliance

460 West 34th Street, New York, NY10001, USA

www.EcoHealthAlliance.org

On Sep 18, 2021, at 10:26 AM, Garry, Robert F b6 wrote:

Of course, the momentum on the lab leak side will continue, with books by Sharri Markison, Alina Chan/Matt Ridley, Op Eds that criticize scientists, 70+ FoIAs by one organization alone, many other FoIAs on their way, 900 pages of FoIA'd grants and reports from EHA/NIAID showing zero evidence of lab leak.

<Screenshot from 2021-09-19 17-04-25.png>

<healthcare-09-01132-v2.pdf>